

Research Article

Kiruthiga Periyannan*, Hemamala Selvaraj, Balachandar Subbu, Muthukrishnan Pallikondaperumal, Ponmurugan Karuppiyah*, Jothi Ramalingam Rajabathar, Hamad Al-Lohedan, and Sadhasivam Thangarasu

Green fabrication of chitosan from marine crustaceans and mushroom waste: Toward sustainable resource utilization

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Abstract: The exoskeletons of crabs, shrimp, and fish are major waste. These wastes contain chitin, an abundant natural polymer found next to cellulose. Thus, disposal of this waste becomes a huge problem for the environment; besides this, reutilization boosts the circular economy. Chitin is partially deacetylated to yield the economically useful product of chitosan and is a heteropolymer. The current study isolated chitosan from mushrooms and various marine crustaceans, i.e., crabs, shrimp, and fish. Chitosan was extracted from marine crustaceans by demineralization, deproteinization, and deacetylation. Later, extracted chitosan was characterized by physicochemical characteristics like deacetylation degree, ash content, protein, color, fat-binding capacity (FBC), water-binding capacity (WBC), pH, and moisture content. The result showed that chitosan yield ranges from 13.0% to 17.0%, the degree of deacetylation range from 82.0% to 85.0%, ash content range from 0.8% to 3.0%, and protein content is below 1.0%. The FBC and WBC range between 320% and 444% and 535% and 602%, respectively. The pH and moisture content range from 7.4 to 8.0 and from 2.0% to 4.0%, respectively. Overall, results specified that crustacean

waste was an exceptional chitosan source with availability and production consistency.

Keywords: chitin, chitosan, demineralization, deproteinization, deacetylation

1 Introduction

Shrimp is an important worldwide fishery product. About 1,377,244 metric tonnes of marine products were exported from 2017 to 2018, of which 65,980 were frozen shrimp. Shrimp processing industries produce 40–50% of their waste in the form of heads and shells. One lakh metric tonnes of shrimp waste are produced in India annually [1]. The shrimp contains 34% to 45% of the head and 10–15% of the body shell. In this waste, there are 35–40% proteins, 10–15% chitin, 10–15% minerals, and 10–15% carotenoids [2]. The shrimp head and shell material are considered bio-waste and have low economic value as it was sold as animal feed [3]. Similarly, crab is considered an important commodity in Indonesia, producing 40–60% of shell waste [4]. In 2014, the amount of canned crab food products exported rose to 28,091 tons, resulting in the yearly creation of one thousand metric tonnes of crab shell waste. If these crab shells are left unchecked, they will create major economic and environmental problems in developing countries.

Fish scales are discarded daily; they are considered important worldwide fishery waste. These fish scales contain 30–40% protein, 20–30% chitosan, and 30–50% calcium carbonate [5]. The enormous amount of fish scales is disposed of as waste. Conversion of these wastes into valuable products is essential. However, the conversion of these scales is rare. In 2017, it was recorded that 15.2 million metric tonnes of crustaceans are produced every year [6]. About 130 million metric tonnes of fish scale waste are discarded as waste every year around the world; degradation of these scales is very slow [7]. Little is used as

* **Corresponding author: Kiruthiga Periyannan**, Department of Microbiology, RVS College of Arts and Science, Coimbatore, Tamil Nadu, India, e-mail: kiruthiga@rvsgroup.com

* **Corresponding author: Ponmurugan Karuppiyah**, Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, 11451, Saudi Arabia, e-mail: ponmurugank@yahoo.com

Hemamala Selvaraj, Balachandar Subbu: Department of Microbiology, RVS College of Arts and Science, Coimbatore, Tamil Nadu, India

Muthukrishnan Pallikondaperumal: Department of Microbiology, P.S.G College of Arts and Science, Coimbatore, 641 014, Tamil Nadu, India

Jothi Ramalingam Rajabathar, Hamad Al-Lohedan: Department of Chemistry, College of Science, King Saud University, Riyadh, 11451, Saudi Arabia

Sadhasivam Thangarasu: Department of Chemical Engineering, Yeungnam University, Gyeongsan, Gyeongbuk, 38541 South Korea

animal feed, and the fertilizers remaining are discarded as waste in the sea, causing global sea pollution. This problem can be solved quickly by recycling this crustacean into commercially important products so that it has prospects. These crustacean shells contain chitin, calcium carbonate, calcium phosphate, proteins, and nitrogen pigments called carotenoids. The concentration of these compounds varies with climatic conditions and species [8]. It is essential to convert this crustacean waste into an economically important product such as chitosan by recycling.

Chitin is an abundant natural polymer found next to cellulose, present in the exoskeleton of crabs, shrimp, mushrooms, fish, worms, insects, and diatoms. Chitosan is partially deacetylated to yield the chitin derivative, chitosan. Chitosan is a heteropolymer of *N*-acetyl D-glucosamine and D-glucosamine joined by beta (1–4) linkage. Demineralization, deproteinization, and deacetylation are the three steps in the chemical extraction of chitosan from crustacean shells. Demineralization is done to dissolve calcium carbonate using acid as a solvent followed by alkaline treatment to dissolve the protein in the shells. Chitin separated during deproteinization can be deacetylated by subjecting it to a high sodium hydroxide concentration of between 50% and 60% and high temperatures between 130°C and 150°C. The degree of acetylation is calculated by the ratio of the above two compounds. Good quality chitosan has a degree of deacetylation (DDA) between 80% and 85%. Chitosan is insoluble in water and is soluble in mild acid [9]. The functional properties of chitosan are antioxidant, antibacterial, flocculant, and filmmaking. Biodegradability, bioactivity, chelating ability, absorption capacity, and environmental friendliness are just a few of chitosan's unique qualities [10]. These properties made chitosan an important compound in aquatic, medical, pharmaceutical, food, agriculture, cosmetic, pulp, and paper industries [11].

Chitosan can be extracted from various sources, such as marine crustaceans, which are mainly found as waste by-products that remain after the processing of marine food products. Recently, chitosan has been extracted from edible mushrooms such as *Agaricus bisporus* stipes with good quality and excellent purity [12]. This study focuses on instead of dumping biowastes such as crab shells, shrimp shells, and fish scales into landfills producing sheer volume to create a nuisance and emitting a bad odor and causing major pollution. It is essential to find alternative methods for its disposal, or it has to be converted to an environmentally important product, chitosan, and also produces chitosan from mushrooms. Extracted chitosan from different sources was characterized by checking its physiological characteristics, such as color, chitosan yield, DDA, ash content, protein

percentage, solubility in 1% acetic acid, fat-binding capacity (FBC), water-binding capacity (WBC), pH, and moisture.

2 Experimental

2.1 Sample collection

Shrimps, crabs, and fish scales were procured from the Sular local fish market in Coimbatore, Tamil Nadu. The scales and shells were separated, cleaned in tap water, and dried for 24 h in an oven at 70°C. The dried shells and scales were crushed, sieved, and then baked for 12 h at 60–70°C to completely dry them out. Mushrooms were purchased from the Sular vegetable market, Coimbatore. Mushrooms were rinsed in water and dried in an oven for 10 days at 50°C. The dried sample was pulverized into a powder and kept aside for later use [13].

2.2 Extraction of chitosan

The chitosan was extracted from crustaceous substance and mushroom using three steps as follows: demineralization, deproteinization, and demineralization.

2.2.1 Deproteinization

Crab shell powder, shrimp shell powder, fish scales, and mushroom powder were deproteinized using 6% NaOH, 4% NaOH, 1% NaOH, and 6.3% NaOH for 2 h at 70°C, 8 h at 80°C, 24 h at 24°C, and 30 min at 95°C, respectively. Deproteinized samples were isolated using filtration and washed using distilled water to neutral pH [14].

2.2.2 Demineralization

Crab shells, shrimp shells, fish scales, and mushrooms were treated with 7.7% HCl for 5 h at 80°C 4% HCl for 12 h at 37°C, 1% HCl for 24 h at 37°C, and 1% acetic acid for 6 h at 95°C, respectively. After demineralization separated fractions were washed with distilled water to neutral pH. Neutralized samples were dried in an oven at 80°C for 45 h [15].

2.2.3 Deacetylation

Chitin from crab, shrimp, fish, and mushroom shells was treated with 50% NaOH at 100°C for 6 h. In order to obtain

chitosan, the deacetylated sample was then neutralized with distilled water and dried in an oven for 12 h at 40°C [14].

2.2.4 Decolorization

1% potassium permanganate solution was used to decolorize isolated chitin for 1 h, followed by 1% oxalic acid for 30 min. The decolorized sample was rinsed in water to achieve a pH of 7 and then dried for 24 h at 60°C in an oven [16].

2.3 Characterization of chitosan

2.3.1 Chitosan yield

The yield of isolated chitosan was estimated by dividing the dry weight of the chitosan by the actual wet weight of the shell waste [17].

$$\text{Yield of chitosan (\%)} = \frac{\text{dry chitosan/sample (g)}}{\times 100} \quad (1)$$

2.3.2 DDA

The DDA was determined using the potentiometric titration method. About 0.5 g was dissolved in 10 mL of 0.1 M HCl solution and was mixed continuously for 30 min. About two drops of phenolphthalein were added to the dissolved chitosan and titrated using 0.1 M sodium hydroxide until the color changed. The deacetylation of the extracted chitosan was determined using the following formula [1]:

$$\text{DD (\%)} = 2.03 \left(V_2 - \frac{V_1}{M} \right) + 0.0042(V_2 - V_1) \quad (2)$$

where M is the mass of the sample, V_2 , V_1 is the volume of 0.1 M sodium hydroxide solution based on deflection points, 2.03 is the coefficient of chitin monomer (weight), and 0.0042 is the efficient difference in molecular weight of chitin and chitosan.

2.3.3 Ash content of isolated chitosan

The ash content of the isolated chitosan was identified by heating 5 g of chitosan until no fumes were produced and again heated at 550°C for 12 h [18].

$$\text{Ash content (\%)} = \frac{\text{Weight of ash}}{\text{Weight of chitosan}} \times 100 \quad (3)$$

2.3.4 Protein present in isolated chitosan

The protein present in the isolated chitosan was determined using the Kjeldahl method [18].

2.3.5 pH of chitosan

About 1 g of isolated chitosan was taken in a 100 mL beaker and 30 mL of distilled water was added and adjusted to neutral pH. Chitosan and water were heated to boiling point for 10 min. The heated chitosan solution was filtered and the filtrate was cooled to room temperature and then pH of the isolated chitosan was estimated using a digital pH meter.

2.3.6 Solubility of chitosan

The solubility of chitosan was measured by dissolving chitosan in 1% of acetic acid solution [19]. Complete mixing can be achieved by stirring the chitosan solution using a magnetic stirrer for 2 h at room temperature. The solution was filtered using Whatman No. 1 filter paper. The filter paper was dried at ambient temperature and re-weighed. The filter paper was weighed before (W_i) and after (W_f) filtration. The percentage of solubility is calculated using the formula:

$$\text{Solubility} = 100 - \left[\left(W_f - \frac{W_i}{W_s} \right) \times 100 \right] \quad (4)$$

where W_f is the final weight of filter paper (g), W_i is the initial weight of filter paper (g), and W_s is the chitosan weight (g).

2.3.7 FBC of isolated chitosan

One gram of chitosan was mixed with 20 mL of soybean oil and incubated for 1 h with periodic shaking for every 10 min for proper mixing. Before incubation, both chitosan and centrifuge tube were weighed separately. After incubation, the chitosan solution was centrifuged at 3,200g for 25 min. The supernatant was discarded, and the pellet weight was calculated by weighing the centrifuge tube with the pellet and subtracting the weight of the centrifuge tube which gives the fat-bound sample weight (pellet weight) [20].

$$\text{Fat binding capacity(\%)} = \frac{\text{Fat bound sample (g)}}{\text{Initial weight of the sample}} \times 100 \quad (5)$$

2.3.8 WBC of isolated chitosan

About 1 g of chitosan was taken in a centrifuge tube. Both the centrifuge tube and chitosan were weighed separately. Chitosan (1 g) was added to 20 mL of distilled water and the solution was mixed and incubated for 1 h which was periodically shaken for proper mixing for every 10 min. After incubation, the chitosan solution was centrifuged at 3,200 rpm for 25 min. The supernatant was discarded and the tube with pellet was weighed [20].

$$\begin{aligned} \text{Water binding capacity(\%)} \\ = \text{Water bound sample (g)} \\ / \text{Initial weight of the sample} \times 100 \end{aligned} \quad (6)$$

2.3.9 Moisture content of chitosan

About 0.5 g of isolated chitosan was placed in a porcelain boat and heated at 105°C for 3 h in the oven and heated chitosan was weighed after 3 h. The moisture content of the chitosan is calculated using the following formula [18]:

$$\% \text{ of moisture content} = w_b - \frac{w_a}{w_b} \times 100 \quad (7)$$

where W_b is the weight of chitosan before heating and W_a is the weight of chitosan after heating.

2.3.10 Purification of chitosan

The isolated chitosan was dissolved in 1% of acetic acid and the dissolved chitosan was precipitated by treating with 4% of NaOH at pH 10. The chitosan purified was stored at room temperature [21].

3 Results and discussion

The chitosan was prepared using biowastes such as crab, shrimp shells, fish scales, and edible mushrooms using three steps: demineralization, deproteinization, and deacetylation (Table 1). In this study, it was found that the color of the crab chitosan is slightly brown which is similar to the results of Sumaila *et al.* [15], that chitosan is off-white in color, odorless, and in the form of semi-crystalline powder. The color of the obtained shrimp chitosan was observed to be off-white which was in congruence, as reported by Mohan *et al.* [22]. The fish scale chitosan color is creamy white which coincides with the results of Muslim *et al.* [23], that white color chitosan obtained from fish scales is similar to chitosan from mushrooms also creamy white (Figure 1). The color of chitosan depends on the sources and effective decoloration.

The yield of chitosan from crab shells is 15.5%, similar to that obtained by Sumaila *et al.* [15], which was 15.4% off-white and odorless from crab shells. Narudin *et al.* [24] reported that chitosan yield from mud crab shells was 16.5%. The yield of chitosan (13%) obtained from mushrooms was similar to that of Margret *et al.* [25]. The chitosan yield from fish scales (17%) result coincides with the result obtained by Srivastav *et al.* [26]. The rate of chitosan extraction depends on the demineralization process. Effective demineralization increases the yield. It was found that the yield of chitosan from fish scales was superior to other sources used in this study (Figure 2).

The DDA is the main parameter in the characterization of chitosan. The process of deacetylation involves the removal of an acetyl group from the chitin molecule. Removal of the acetyl group is difficult therefore deacetylation process needs a high concentration of NaOH. The DDA affects the physicochemical property of chitosan.

Table 1: Extraction of chitosan from mushroom and different marine crustaceans

Characters	Crab	Shrimp	Fish scales	Mushroom
Deproteinization	1.25 M (6%) NaOH for 2 h at 70°C	4% NaOH 8 h at 80°C	1% NaOH 24 h at 25°C	1 M (6.25%) NaOH 30 min at 95°C
Washed to neutral	Water	Water	Water	Water
Demineralization	1.25 M (7.7%) HCl for 5 h at 80°C	4% HCl for 12 h at 37°C	1% HCl for 24 h at 37°C	2% acetic acid for 6 h at 95°C
Washed to neutral	Water	Water	Water	Water
Deacetylation	50% NaOH for 6 h at 80°C	50% NaOH for 6 h at 80°C	50% NaOH for 6 h at 80°C	50% NaOH for 6 h at 80°C
Washed to neutral	Water	Water	Water	Water
Decolorization	1% potassium permanganate for 1 h and 1% oxalic acid	1% potassium permanganate for 1 h and 1% oxalic acid	1% potassium permanganate for 1 h and 1% oxalic acid	1% potassium permanganate for 1 h and 1% oxalic acid



Figure 1: Chitosan from different sources: (a) crab, (b) shrimp, (c) fish, and (d) mushroom.

The commercial chitosan DDA ranges from 66% to 95% [11]. The deacetylated chitosan used to be soluble in low concentrations of acid. The DDA of chitosan from crab is 85%; this obtained result is similar to the DDA of crab (82%) by Pambudi et al. [27]. DDA of fish scales chitosan is higher than the result obtained by Molina-Ramírez et al. [28]. DDA chitosan isolated from the mushroom is 79% higher than Fadhil and Mous's results [14]. The obtained results were in the standard range of deacetylation of chitosan (Figure 3).

The ash content of chitosan is an important parameter to determine the quality of chitosan. Ash content depends on effective demineralization. Demineralization removes calcium carbonate from crustaceans. Good quality chitosan must have an ash content less than 1%. The ash content of chitosan isolated from crab, shrimp, fish scales, and mushroom range from 1% to 3% (Figure 4). It shows that minerals in the crustaceans' sample are partially recovered, and the demineralization process could be better. The results of Karnila et al. [29] coincide with the obtained results. It states that ash content can be high, up to 17%. The obtained ash content of chitosan from fish scales

(Table 2) is similar to the results of Ooi et al. [30], showing that the ash content of chitosan obtained from crab samples is 1.8–3.2%; this result coincides with the present study result, showing that chitosan from crab sample is 2%. The ash content of shrimp chitosan is 0.80% which is similar to the result reported by Renuka et al. [31]. High ash content is due to inefficient washing and demineralization steps. High ash content will affect the solubility and viscosity.

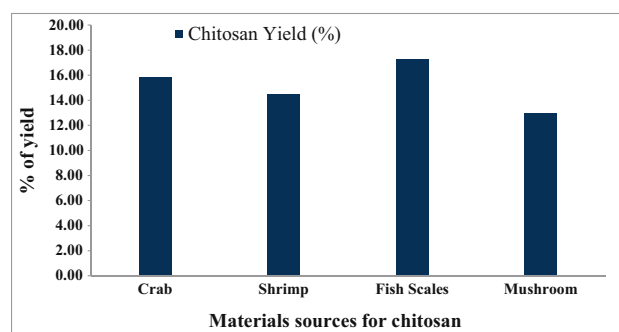


Figure 2: Percentage of yield from different materials source.

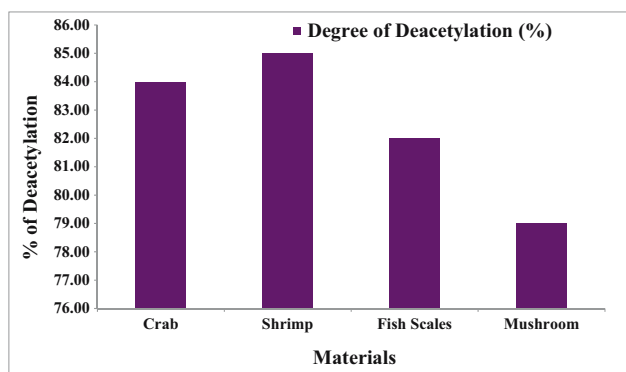


Figure 3: Percentage of DDA.

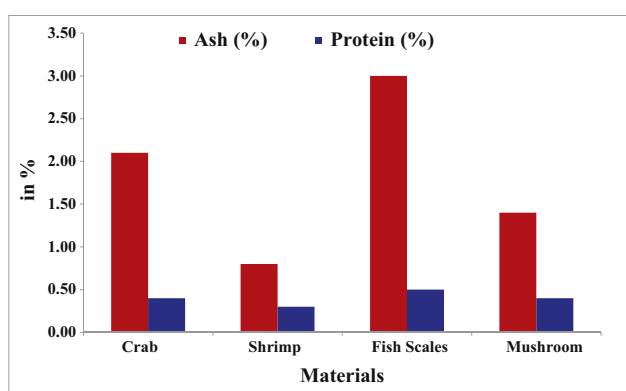


Figure 4: Percentage of ash and protein content.

In this study, ash content was in the optimum range; hence, proper demineralization and washing were evident.

The protein in the shrimp chitosan is 0.4%, lower than the result (1.99%) reported by Mohan *et al.* [22]. The protein in the crab chitosan is 0.4% and the obtained results coincide with the results of Jabeur *et al.* [32]. The protein in the fish scale chitosan is 0.5%, which is lower than Caudhry *et al.*'s results [33]. The protein present in the mushroom

sample is 0.4% (Figure 4). Lower protein content indicates effective demineralization.

The solubility of chitosan depends on the quality of chitosan. Good quality chitosan has higher solubility. The solubility of chitosan depends on the DDA because solubility is related to the removal of an acetyl group from chitin (Table 2). Lower solubility value shows incomplete removal of protein and acetyl group. Chitosan is soluble in inorganic acids because it is a highly protonated free amino group that attracts ionic compounds. Obtained shrimp chitosan is 98% soluble which is similar to results reported by Renuka *et al.* [31]. Solubility indicates the purity of isolated chitosan. The solubility of chitosan from crab is 97%, similar to the results of Demir *et al.* [34]. Lower solubility is due to the incomplete removal of protein and acetyl group in the chitosan.

The FBC of chitosan depends on the viscosity of the chitosan. If the chitosan has a lower viscosity, it will also have a lower FBC. FBC of chitosan from fish scales, crab, shrimp, and mushroom ranges from 320 to 444. The FBC of chitosan from crab sample is 333.4%, which is similar to the result reported by Demir *et al.* [34]. Various steps involved in the extraction may affect the chitosan FBC. High fat binding was observed when demineralization was observed by deproteinization and deacetylation, while FBC decreased when deproteinization was followed by demineralization and deacetylation. The FBC of shrimp chitosan is 444%, which is similar to the result reported by Abirami *et al.* [35]. The FBC of fish scale chitosan is 325%, which is slightly lower than the result observed by Srivastava *et al.* [26], and the FBC of mushroom chitosan is 320%, which is slightly higher than the result reported by Fadhil and Mous [14]. FBC of the commercially available chitosan is in the range of 314–535% and the chitosan produced in the present study coincides with commercially available chitosan (Figure 5).

WBC depends on salt-forming groups' availability, crystallinity dissimilarities, and residual protein WBC of fish

Table 2: The physiological characteristics of chitosan isolated from different samples

Parameters	Crab	Shrimp	Fish scales	Mushroom
Color	Slight brownly white	White	Creamy white	Creamy white
Chitosan yield (%)	15.5	15.20	17	13
DDA (%)	84	85	82	79
Ash content (%)	2.1	0.80	3	1.4
Protein (%)	0.4	0.3	0.5	0.4
Solubility in 1% acetic acid	Soluble 97%	Soluble 98%	Soluble 95%	Soluble 91%
FBC	333.4	444.1	325	320
WBC	554	602.2	535	635
pH	7.5	8	7.7	7.4
Moisture content	3	2.01	3	4

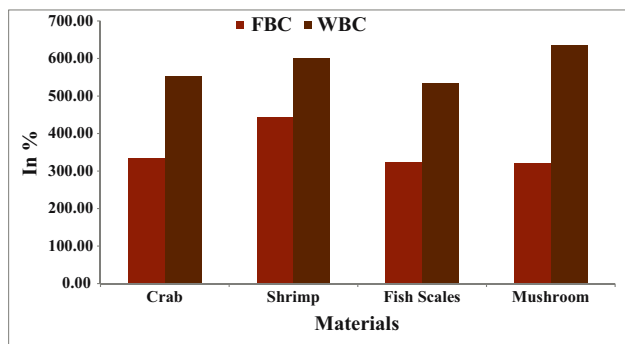


Figure 5: Percentage of fat binding and water binding properties.

scales, crab, shrimp, and mushrooms ranging from 535% to 602% (Table 1). The WBC of crab sample is identified as 554%, which is similar to the result reported by Demir et al. [34], and the WBC of shrimp chitosan is 602.2%, which coincides with the result observed by Reunka et al. [31]. The WBC of fish scale chitosan is 535% observed result coincides with the result of Srivastav et al. [26], and the WBC of mushroom chitosan (635%) was reported by Fadhil and Mouis [14]. Commercially available chitosan has WBC in the range of 458–805%. The WBC of obtained chitosan was also in the similar range of commercially available chitosan (Figure 5).

The pH of the extracted chitosan ranges from 7.4 to 8.0 pH of the crab chitosan is 7.5, as reported by the results of Olafadehan et al. [36], was 6.8 to 7.5 pH of shrimp chitosan is 8.0 this is similar to the results of observed by Reunka et al. [31] (7.9), pH of fish scale chitosan is 7.7 obtained result coincides with the results of Gokulalakshmi et al. [7] (pH 7.0), and the pH of mushroom chitosan is 7.4 which is slightly lower than the result obtained by Mythil and Aysha [37] (Figure 6).

The moisture content of chitosan isolated from crustaceans ranges from 2% to 4% (Figure 7). Generally, good quality chitosan must have a moisture content of at most

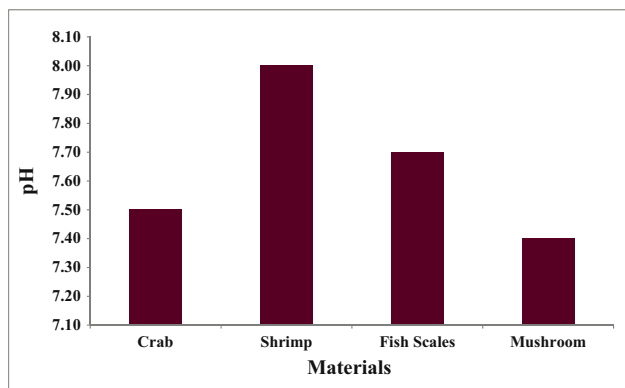


Figure 6: pH of the extracted chitosan from different materials.

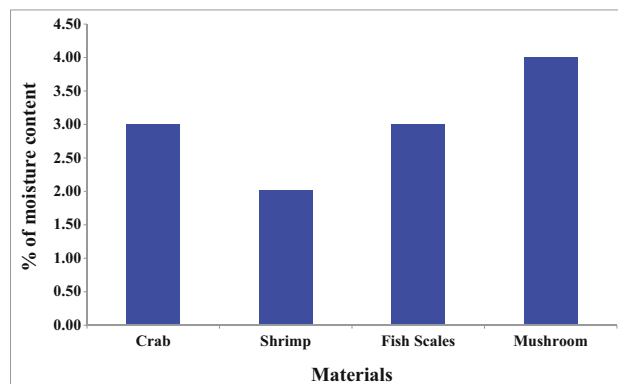


Figure 7: Percentage of moisture content of extracted chitosan from different materials.

5% [38]. The moisture content of crab chitosan is 3% and the obtained result coincides with the results of Gaikwad et al. [39], reporting that the moisture content of crab is 2.37 and 5.4% and similar to the results of Sumaila et al. [15]. The moisture content of shrimp chitosan is 2.01%. It is similar to the results of Hossain and Iqbal [40] (1.5% and 1.8%). The moisture content of fish scale chitosan is 3%, which coincides with the results of Takarina and Fanani [41]. The moisture content of mushroom chitosan is 4% which is lower than the moisture content reported by Hossain et al. [12]. Commercial chitosan showed less than 10% of moisture [42]. The high-water content will damage the polymer structure of chitosan through hydrolysis. The moisture content of chitosan also depends on the surrounding sunlight intensity and relative humidity. Chitosan can observe more water during storage due to its hygroscopic nature.

4 Conclusion

This study presents a green and sustainable fabrication method for producing high-value chitosan from fishery wastes, such as crab, prawns, fish scales, and mushrooms. The deacetylation technique successfully transformed chitin extracted from these waste sources into chitosan, a versatile biopolymer with numerous applications.

The results revealed that fish scales exhibited the highest chitosan yield among all the sources investigated. Additionally, shrimp shells proved to be an effective and promising resource for obtaining high levels of deacetylated chitosan. These findings underscore the potential of utilizing these abundant seafood by-products for chitosan production, thereby contributing to waste reduction and promoting a more sustainable approach in the seafood

industry. Furthermore, assessing chitosan's quality, considering its proximate composition including ash content, protein content, solubility, FBC, WBC, pH, and moisture content, yielded promising results. The chitosan derived from shrimp shells demonstrated high solubility and exhibited comparable FBC, WBC, and pH characteristics to other high-quality chitosan samples.

This research validates the viability of the proposed green fabrication method and highlights the potential of specific waste sources, such as fish scales and shrimp shells, for obtaining superior chitosan yields with desirable properties. The findings pave the way for developing more sustainable and economically viable processes to produce high-value chitosan, offering diverse applications in various industries, including pharmaceuticals, food, agriculture, and wastewater treatment. Nevertheless, further studies and optimization of the deacetylation process are recommended to continuously enhance chitosan yield and quality. Additionally, exploring novel applications for chitosan and evaluating its performance in various practical scenarios would provide valuable insights for commercial-scale utilization.

Overall, this research contributes significantly to the advancement of green synthesis methods for chitosan production and highlights the importance of valorizing fishery waste as a valuable resource for generating high-value products, thus promoting the principles of a circular and sustainable economy.

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Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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