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Valorization of crustacean shell waste for extraction of chitin and chitosan

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Abstract
The crustacean processing industry generates a huge quantity of by-products that can be utilized as a key source of chitin, chitosan, oligochitosan, and other functional marine-biopolymer. The present study uses crustacean shell waste to extract chitin and chitosan. Chitin and chitosan extraction was executed through chemical processes by comprising demineralization, deproteinization, and deacetylation step with three sub-sets of treatment (T1, T2, and T3). Chitosan purity was tested for characterization of chitosan by determining the ash content, moisture content, elemental analysis, solubility test. Results show that T3 (1.5N HCL/NaOH) treatment gives better yield as well as a high degree of solubility as compared to other treatment groups. Productions of chitin and chitosan from crustacean shell waste will reduce the environmental impact on waste management as well as create employment and, export opportunities.

Keywords: crustacean shell waste, chitin, chitosan, extraction, evaluation

1. Introduction
Globally, millions of tons of crustaceans like prawn, shrimp, crab, lobster, crayfish, etc. are produced and consumed as a rich source of seafood protein every year. The external shells of crustaceans constitute about half their body mass are usually discarded as waste. These discarded crustacean shells are a prominent source of chitin, chitosan, and protein, but pose problems for the ecosystem, primarily due to negligible solubility in water, and a very slow biodegradation rate (Muley *et al*., 2018) [12].

Chitin is a naturally occurring polysaccharide mainly found in the cell walls of crustacean and insect exoskeletons and is considered as second most abundant marine biopolymer in nature (Samar *et al*., 2013, Sojitra *et al*., 2017) [19, 21]. Chitin's unique properties, such as biocompatibility, non-toxicity, biodegradability, and adsorption properties make its wider application in the food, pharmaceutical, and biomedical industry. Chitin can be readily obtained by simple extraction method and the major source of industrial chitin is derived from wastes of marine crustacean shells, e.g., shrimp, crab, or krill shells. The crustacean shell is composed of 30–40% proteins, 30–50% mineral salts, and 13–42% chitin occurring in alpha, beta, and gamma forms. In the processing of shrimps and prawns for human consumption, between 40 to 50% of the total mass is waste and 40% of this waste is considered as source chitin (Nadar *et al*., 2016) [14].

Chitosan is obtained on substantial deacetylation through alkaline treatment of chitin under different conditions. The isolation of chitosan from prawn shells comprises the removal of proteins, calcium carbonate, pigment, and acetyl groups (Mohammed *et al*., 2013) [10]. The quality and properties of chitosan are primarily associated with its moisture content, degree of deacetylation, molecular weight, solubility in different solvent systems. These properties are mainly influenced by the extraction procedure, time-temperature combinations, different strength and the ratio of alkali used, source of raw chitin and its particle size, rate of agitation process, shear stress, and previous processing treatments used in chitin extraction (Ramasamy *et al*., 2017) [17]. Chitosan is gaining tremendous focus as a functional biopolymer because of its distinct applications in numerous fields (Muley *et al*., 2018 and Mohanasrinivasan *et al*., 2014) [12, 11]. It can be used as a therapeutic agent because it has antibacterial and antifungal characteristics, making it interesting for applications in agriculture, medicine, the environment, and the food, cosmetic, and textile industries (Srbová *et al*., 2016) [22].

Several processes have been proposed to use crustacean waste for the extraction of chitin and chitosan.

Chitin extraction involves alkaline extraction of organics and acid solubilization/decomposition of minerals, after protein removal. During such extractions, the chitin molecule also suffers some structural changes, including a moderate degree of deacetylation (Paul *et al*., 2014) [16]. Research interest in the area of renewable marine crustacean waste has gained tremendous attention in recent years. Therefore, the present study focus on the complete utilization of marine crustacean shell waste to extract low coast chitin and chitosan marine biopolymer to use it as a key material for many industrial applications.

2. Materials and Methods

2.1 Collection of raw materials

Crustacean shell waste with slight adherence of meat was collected from local fish markets of Paralakhemundi, Odisha, India, and used for chitin and chitosan extraction. All the chemicals and reagents (Sodium hydroxide, hydrochloric acid, acetic acid (CH3COOH), De-ionized water) were used for the research work are analytical grade, and procured from reliable sources.

2.2 Preparation crustacean shell waste

Shrimp-Prawn shell waste (carapace, body shell, claw) was washed under running tap water to remove the soluble organics, adherent proteins, and other impurities. Thereafter, shells were boiled in water (water: shell: 3:1) for 1 h to remove the tissue and kept in an oven at 160°C for 2 h to make them more brittle and cut into small pieces and pulverized by using a grinder.

2.3 Extraction methods of chitin and chitosan

The extraction of the chitin and chitosan from crustacean shells was carried out by chemical treatment with some modifications of Ahing & Wid (2016) [2] and Black & Schwartz, (1950) [4]. The major procedure for obtaining chitosan is based on the alkaline deacetylation of chitin with a strongly alkaline solution at different periods.

2.3.1 Demineralization process

The demineralization was carried out by weight 3 gm of crustacean shell powder by using HCl (1N (T1), 1.25N (T2), 1.5N (T3)) with the ratio of 14ml: 1g (w/v) at room temperature for 24 h. The obtained product was washed to neutralize under the running tap water. Finally, solid particle ware collected and washed by using distilled water and kept for vacuum drying.

2.3.2 Deproteinization process

The deproteinization was occurred by heating 3 gm of crustacean shell powder after adding 5% NaOH (1N (T1), 1.25N (T2), 1.5N (T3)) with the ratio of 12ml: 1g (w/v) at 90˚C for 24 h. The product obtained was neutralized by washing under running tap water. The solid particles were collected and again washed with distilled water. Finally, the solid products were dried in a vacuum dryer, then weigh and packed in an air-tight polyethylene pack.

2.3.3 Deacetylation process

Then the demineralized product was treated with 50% NaOH (T1, T2, T3) with the ratio of 14ml: 1g (w/v) at room temperature for 75 h. with stirring. The obtained deacetylated solid part was filtered then collected and washed using distilled water. Finally, the deacetylated products were dried in a vacuum dryer, followed by weighing and packaging.

2.4 Proximate composition, yield, moisture, ash, elemental analysis, solubility, and insoluble matter of crustacean shell wastes

The proximate composition of shrimp and prawn shell waste was determined by measuring the moisture, crude protein, chitin nitrogen, and ash content according to the standard methods of AOAC (1975) [3]. The moisture content was determined by drying the sample in an oven at 100 ± 5°C until a constant weight was obtained. Total nitrogen (TN) content was determined with the Kjeldahl method, and actual protein content was calculated by multiplying protein nitrogen value with conversion factor (PN×6.25). Ash content was measured by dry ashing the sample in a muffle furnace at 550°C for 8h. To test solubility, 1 g of chitin and chitosan were weighed and dissolved in 100 mL of 1% acetic acid solution. The mixture was then stirred well and used to keep for 2hrs at ambient temperature. The mixture was subsequently passed through a pre-weighed filter paper (Whatman No.1) and the filter paper was dried and re-weighed upon completion of the filtration. The percentage of solubility characteristic was calculated from the ratio of weight gain of filter paper x100. An elemental CHNS analyzer was used to measure the ratio of carbon, hydrogen, nitrogen, and sulfate content.

2.5 Statistical analysis

Analysis of variance (ANOVA) was carried out, and Tukeys HSD determined the significant difference among the treatments group of chitin and chitosan extraction. The level of significance was set up at $p \leq 0.05$. All the above experiments were carried out in triplicates and the results were expressed as a Mean ± Standard deviation.

3. Results and Discussion

The result of the extraction of chitin and chitosan via deproteinization, demineralization, and deacetylation from crustacean shells waste is a preliminary study to evaluate the quality of extracted chitin and chitosan. The extraction methods followed the three key stages including demineralization, deproteinization, and deacetylation with each method there was three sub-set of treatments (Figure 1).

Fig 1: Steps for extraction of chitin and chitosan from crustacean shell waste

3.1 Proximate composition, elemental analysis, and yield of chitin and chitosan

The proximate composition of the raw crustacean shell waste, as well as chitin and chitosan, was evaluated and it shows significant variation in protein, moisture, ash content of the sample. The protein content of the raw shrimp shell was high (36.40 ± 1.10) as compared to the extracted chitin and chitosan (1.25 ± 0.05 and 1.15 ± 0.05). Similar kinds of results were also reported by (Saravana *et al*., 2018). [20] The moisture content was found to be 12.50 ± 1.05, 4.50 ± 0.02, and 1.30 ± 0.02 for raw crustacean shells, chitin, and chitosan respectively, which was similar to that of the results reported by (Saravana *et al*., 2018) [20]. Several research groups (Mohanasrinivasan *et al*., 2014, and Hossain & Iqbal, 2014) [11, 5] have reported 1–1.30% moisture content of chitosan extracted from brine shrimp shells waste. Although Li *et al*., (2020) [9] reported that commercial chitosan products may contain >10% moisture content. Moisture content affects the quality of chitosan produced from co-products. Szymanska and Winnicka, (2015) [24] also suggested that the moisture content of chitosan must be low to prevent damage to the polymer. Chitosan is hygroscopic which could lead to stickiness and also propel fungal growth. Lower moisture content in chitosan may lead to better storage stability and hence better quality (Subhapradha *et al*., 2013) [23]. The raw crustacean shell, extracted chitin and chitosan had an ash content of 31.75 ± 1.10, 0.45 ± 0.05, and 0.40 ± 0.02 respectively, which is in the range reported for commercial chitin and chitosan. Ash content is an indicator of the purity and efficacy of the demineralization step during extraction and is primarily dependent on the starting material and its composition. The presence of minerals even after precipitation and filtration during chitosan extraction is due to their association with chitosan molecules. The presence of ash in chitosan could prominently affect its molecular weight, solubility, and viscosity in different solvents (Subhapradha *et al*., 2013) [23]. Islam, Khan & Alam, (2016) [6], reported that the ash content was 1.5% on the dry wet basis in shrimp shells which is similar to the present study. The elemental CHNS analysis revealed the amount of carbon, nitrogen, and hydrogen in the extracted sample. The amount of carbon, nitrogen and hydrogen is extracted chitin and chitosan was 45.60 & 36.57, 8.90 & 6.50, 6.60 & 5.50%, and 0.20 & 0.15 respectively. The carbon, hydrogen, nitrogen, and sulfate content of chitin and chitosan generally depend on the raw material source, and conditions used for extraction of chitin and chitosan (Kaya *et al*., 2014) [7].

Table 1: Proximate composition of raw crustacean shell waste, chitin, and chitosan

Parameters	Raw crustacean shell waste	Chitin	Chitosan
Moisture (%)	12.50 ± 1.05	4.50 ± 0.02	1.30 ± 0.02
Crude protein (%)	36.40 ± 1.10	1.25 ± 0.05	1.15 ± 0.05
Ash (%)	31.75 ± 1.10	0.45 ± 0.05	0.40 ± 0.02

3.2 Characterization of chitin and chitosan (Yield, solubility and insoluble matter, colour)

The chitin and chitosan yield extracted from the crustacean shell was 22.45±0.08 (T1) 22.75±0.05 (T2), 23.15±0.10 (T3) and 15.20±0.10 (T1), 15.80±0.12 (T2), 17.20±0.15 (T3) respectively, which was following the yields reported by (Mohammed *et al*., 2013). The percent yield is associated with the effectiveness in the removal of minerals and proteins attached therewith. Abdulkarim *et al*., (2013) **[1]** reported a 15% yield of chitosan from shrimp shell waste which is slightly lower than the yield of chitosan in the present study. The variation could be due to the difference in the methodology of extraction and age of the crustacean species like shrimp and prawn from which the sample was taken.

In the present study, the chitosan solubility rate was 99.0±2.95 (T1), 99.2±2.85 (T2) 99.4±1.80 (T3) respectively, which is similar to Ahing and Wid (2016).**[2]** Chitosan was completely soluble in 1% acetic acid with 2.70±0.10% insoluble matter while it was moderately soluble in 1% lactic acid having 7.5±0.10% insoluble matter. Chitosan was entirely insoluble in water as well as 1% hydrochloric, phosphoric, and sulphuric acid, respectively, and the insoluble matter was more than 90%. The solubility of chitosan was tested using organic acids like acetic, lactic, and formic acid

mainly due to the ease of protonation of the amino group in chitosan at a low pH environment. Chitosan solubility is primarily associated with its molecular weight, DDA, chitosan source, the pattern of substitution of its monomers i.e. N-acetyl-D-glucosamine and D-glucosamine, ratio and concentration between acid and chitosan (Rui *et al*., 2017) [18]. The proteinaceous impurities left after the deproteination process during chitosan extraction also have a substantial effect on its solubility in water and several organic and inorganic acids (Muley *et al*., 2015) [13]. The alterations of chitosan solubility in different acids may be due to fluctuations in the combination of time and temperature applied during the deacetylation step, ratio and strength of alkali/acid, and the nature and particle size of chitin used during extraction processes (Kumari, Suneeta, *et al*. 2017) [8]. Generally, chitin and chitosan were visually seen as creamish yellow to white color, while its L*, a*, and b* values were found to be 42.50±0.25&45.0±0.20, 1.40±0.05& 1.27±0.02 and 6.5±0.04 &6.0±0.02 respectively, in the colorimeter analysis. The colorimeter L* value represents lightness and darkness, a* value represents redness-greenness, while b* value indicates yellowness-blueness of substances. A transparent solution of chitosan was formed after dissolving in 1% acetic acid. The color and whiteness index is affected by the alkaline treatment and extraction time applied for chitin and chitosan extraction. The color of chitin and chitosan extracted from the present study is similar to the chitin and chitosan obtained from Naznin *et al*., (2005) [15].

Table 2: Characterization of chitin and chitosan deriving from crustacean shell waste

Treatment (HCl, NaOH Concentration)	Chitin yield (%)	Chitosan yield (%)	Colour of chitin	Colour of chitosan	Chitosan Solubility test (%)
T1 (1N)	22.45±0.08	15.20±0.10	Pale pink colour	White	99.0±2.95
T 2 (1.25N)	22.75±0.05	15.80±0.12	Pale pink colour	Brilliant white	99.2±2.85
T3 (1.5N)	23.15±0.10	17.20±0.15	Dim pink colour	Super white	99.4±1.80

4. Conclusions

A vast amount of waste produced from crustacean processing industries can be a vital source of many useful bio-functional substances including chitin and chitosan. In this present study, marine crustacean shell waste was used to obtain chitin and chitosan by improvised different treatment techniques which are more convenient according to the handling procedure, time, yield, and accuracy of chitosan production. Complete valorization of crustacean shells to extract chitin and chitosan will reduce the environmental impact on seafood waste management as well as create employment and, exporting potential. So there is a need to improve research utilization of seafood waste through the inclusion of more advanced technological inputs in near future.

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