

Comparison of the Quality of Chitin and Chitosan from Shrimp, Crab and Squilla Waste

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Abstract

Chitin and Chitosan obtained from the crustaceans are having more biological value such as physiological compatibility, non-toxicity, bio digestibility, adsorption and chelating capacity. These biological values of chitosan depend on the quality parameters which are directly related to the source of the raw material. In this study, three commercially available crustacean shell waste such as shrimp, crab and squilla were used for the extraction of chitin and chitosan. The chemical treatment of demineralization, deproteinization and deacetylation were used for the production of chitosan. The viscosity quality parameter of the shrimp chitosan (5300cPs) was better than the crab and squilla chitosan. It is due the high solubility (97.65%) of the shrimp chitosan in 1% acetic acid. The degree of deacetylation of the shrimp chitosan (81.24%) directly relates the solubility of the chitosan. The chitosan with these quality parameters considered to be the excellent biological value. The yield of shrimp chitosan (15.4%) was also more when compare to crab and squilla chitosan. These result showed that utilisation of shrimp shell waste for the production of chitin and chitosan will give more economical and biological value along with reduction of environmental pollution.



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Introduction

World population growth continuously overtakes the rate of fish production. The FAO Code of Conduct for Responsible Fisheries was concerned about the clear signs of over-exploitation of fish stocks and to recommend new approaches to fisheries management which included conservation, environmental, social, and economic considerations¹⁴.

The quantity of processing waste generated around 20-80% depends on the species, processing stage, and the technology used⁹. The Indian seafood export was Rs. 9.5 lakhs ton for the year 2015-16 dominated by crustaceans and molluscs¹⁹. The industries reject approximately up to 75 % of total weight of raw material, these can create serious pollution and disposal problems⁹. The crustacean shell wastes

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obtained from seafood industries have only a low economic value and they are used as either animal feed or organic manure²⁶. Squilla is another underutilized trash fish contributes to an estimated quantity of 1.8% of the total marine landings of India and converting into fishmeal, poultry feed and manure²⁷. The shellfish waste contains 8- 10 % chitin, 30-65 % protein 10-20 % and calcium on a dry weight basis²³. Chitin considered as the second most abundant biopolymer after cellulose found in the nature²². Chitosan has attracted consideration due to the unique cationic nature, which is obtained after the process called N- deacetylation of chitin²⁸. Chitin and chitosan are commercial attracted due to their physiological compatibility, non-toxicity, bio digestibility, and adsorption and chelating capacity. These characteristics of chitosan have many biotechnological industrial applications such as clinical, cosmetics, food, pharmaceutical, agriculture, aquaculture and environmental engineering²⁴. The type and quality of chitin and chitosan varies with different source, though different concentration of wide range of acid and alkali are used to extract chitin and chitosan from shell waste¹³. Nguyen Van Toan²⁸ reported that the chitin and chitosan produced with 3 % HCl for demineralisation, 4% NaOH for deproteination and 50 % NaOH for deacetylation was resulted in good quality chitin and chitosan. By adopting Nguyen Van Toan²⁸ chemical method, this study was designed to compare the quality of chitin and chitosan obtained from shrimp, crab and squilla using this processing method.

Materials and Methods

Fresh local Indian white shrimp (*Fenneropenaeus indicus*), Mud crab (*Scylla serrata*) and Squilla (*Squilla leptoquill*) shells were collected from Pulicat lake, Tamil Nadu, India. The proximate composition of the fresh shells was estimated on wet weight basis. The shell wastes were sun dried after cleaning. The dried shells were packed in sealed polythene bags and kept at ambient temperature (28±2 °C) to carry out the experiments.

Composition Analysis

The proximate composition of crustacean shell waste was estimated by standard protocols as follows; moisture, protein, ash⁵ and lipid⁷. There were three replicates sampling procedure adapted for the

analyses various parameters. The percentage of moisture content was estimated by dehydration of sample (approx. 5g) in an oven at 105 °C for 24 hours. For the estimation of crude protein, around 1g of shell waste sample was used to estimate the nitrogen content in semi-automatic Kjeldahl apparatus. The percentage of crude protein calculated from the total nitrogen by the multiplication factor of 6.25. Ash content was estimated using muffle furnace by burning the shell sample at 550 °C for 6 hours. The crude lipid content was estimated using Soxhlet apparatus by extracting with the solvent acetone from the known quantity of dried shell waste. Analyses were made in three replicates.

Preparation of Chitin and Chitosan

The preparation of chitin was followed by 2 (two) treatment steps namely demineralization, deproteinization and the production of chitosan by the additional treatment step called deacetylation. The schematic diagram for the above processing steps are explained below and shown in Fig.1.

Demineralization

Demineralization of shell wastes were treated with 3% HCl with a solvent to solid ratio 5:1 (v/w) at room temperature (28±2 °C) for 16 hrs²⁸. The residual HCl was removed by repeated washing by portable water to reach the neutral pH.

Deproteinization

After demineralization, 4% NaOH with a solvent to solid ratio 5:1 (v/w) for 20 hours at ambient temperature (28±2 °C) carried out for the deproteinization of shells²⁸. The residual NaOH was removed by repeated washing by portable water to reach the neutral pH. The filtered chitin was dehydrated and made in to powder to enable deacetylation process.

Deacetylation

Removal of acetyl groups from chitin obtained from the shell wastes were treated with 50 % NaOH with a solvent to solid ratio 10:1 (v/w) for 20 hours at 65 °C temperature²⁸. The residual NaOH was removed by repeated washing by portable water to reach the neutral pH. The filtered chitosan was dehydrated at hot air oven for 4 hours at 65±50 °C to enable the characterization.

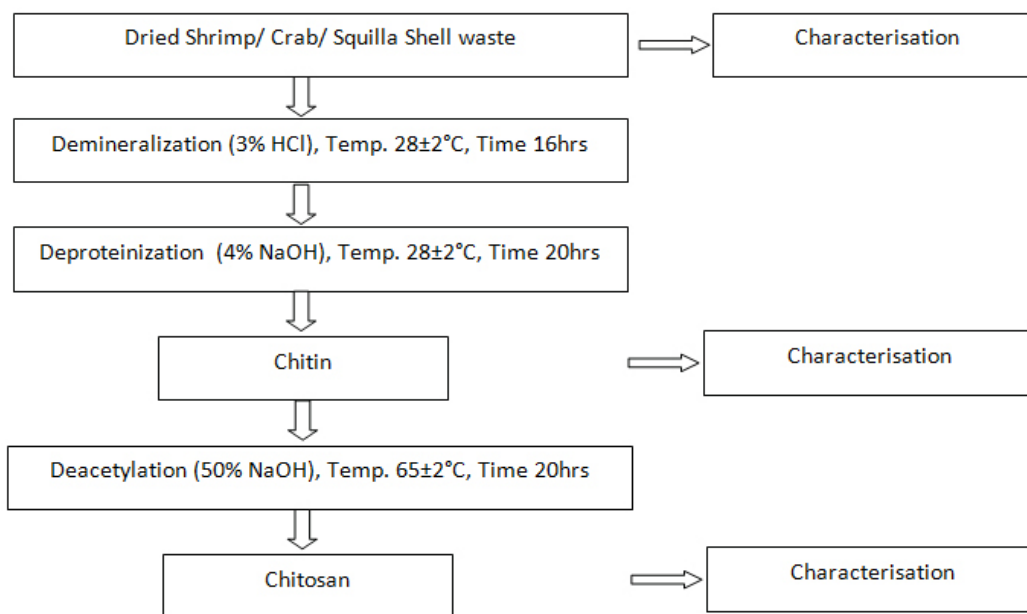


Fig. 1: Schematic flow diagram for production of chitin and chitosan

Determination of Degree of Deacetylation

The acid-base titration method was carried out to estimate the degree of deacetylation (DD)¹³. Around 0.1g of chitosan was dissolved in 30 ml of 0.1 M HCl solution with 5–6 drops of methyl orange indicator at room temperature. The red coloured reaction mixture was titrated with standardised 0.1 mol NaOH solution until it turned to orange colour. The formula used for calculating DD% as follows

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

Where, C_1 = (mol/l) concentration of standard HCl acid solution, C_2 = (mol/l) concentration of standard NaOH solution, V_1 = (ml) volume of the standard HCl solution, V_2 = (ml) volume of standard NaOH solution, and M = (g) weight of chitosan. 0.016 = gram equivalent weight of amino (NH_2) group in 1 ml of standard 1 mol/l HCl acid solution and 0.0994 = the proportion of amino (NH_2) group by weight in chitosan. The Degree of acetylation was calculated by subtracting the value of degree of deacetylation from 100%.

Solubility

Unlike chitosan, chitin is insoluble in organic solvents but chitosan is soluble in acidic condition below pH 6.0. organic acids such as acetic, formic and lactic

acids can solubilise the chitosan. But, 1 % acetic acid solution at pH 4.0 is most commonly used as a reference solution to solubilise chitosan. The higher concentration of acetic acid solutions at very high temperature leads to the depolymerization of chitosan. Usage of inorganic acid for dissolving chitosan is very limited⁶.

Viscosity

Viscosity of chitosan was estimated with a viscometer model ML-98965-40, Cole - Parmer, Vernon Hills, IL 60061, US. The 1% chitosan solution was prepared using 1% acetic acid on a dry basis was used to measure the viscosity. The viscometer fixed with No. 5 spindle and operated at 50 rpm at 25 °C on 1% solutions. Analyses were made in three replicates. The values were reported in centipoises (cPs) units.

Results

The table 1 shows the proximate composition value of moisture, ash, crude protein and crude fat content of various shell waste used in this experiments. The yield of the shell waste explains the quantity of shell waste generated from the crustacean during seafood processing. The shell waste obtained from crab shows the maximum yield of 55.58±0.18 %.

Table 1: Analysis of shell wastes

Shell waste	Yield(%)	Parameter (%)			
		Moisture	Ash	Protein	Fat
Shrimp	45.14±0.15	21.51±0.13	20.45±0.01	32.13±0.08	9.13
Crab	55.58±0.18	20.57±0.08	25.43±0.03	30.41±0.05	10.14
Squilla	52.26±0.09	22.59±0.19	22.56±0.02	33.18±0.10	9.82

The table 2 explains the composition of chitin obtained from shrimp, crab and squilla shell waste. The result on chitin yield shows that shrimp waste

can produce more yield of 17.36±0.07 % when compare to crab and squilla. The crude fat content of chitin was not detectable level for all shell waste.

Table 2: Analysis of chitin

Chitin	Yield(%)	Parameter (%)			
		Moisture	Ash	Protein	Fat
Shrimp	17.36±0.07	8.31±0.11	0.48±0.02	0.80 ±0.15	ND
Crab	14.52±0.12	9.32±0.13	0.96 ±0.01	0.82±0.12	ND
Squilla	13.45±0.21	9.10±0.16	0.76±0.03	0.87±0.09	ND

ND: Not detectable

The composition and quality parameters of the chitosan were showed in table 3. The solubility of chitosan obtained from shrimp is very high (97.02±0.24 %) which is the best value among the

waste involved in the experiment. The solubility of the chitosan influenced by the degree of deacetylation (71.58 ±0.09%), which exhibit the better viscosity 5300±32 cps value.

Table 3: Analysis of chitosan

Chitosan	Yield (%)	Parameter (%)						Viscosity (cps)
		Moisture	Ash	Fat	solubility	DA	DD	
Shrimp	15.40 ±0.16	7.56 ±0.07	0.36 ±0.01	ND	97.02 ±0.24	28.52 ±0.07	71.58 ±0.09	5300 ±32
Crab	13.25 ±0.09	7.62 ±0.14	0.76 ±0.02	ND	85.25 ±0.31	36.54 ±0.09	63.53 ±0.10	465 ±15
Squilla	12.56 ±0.14	7.67 ±0.12	0.58 ±0.1	ND	89.65 ±0.25	34.57 ±0.11	65.54 ±0.06	2600 ±34

Note: DA - Degree of acetylation, DD- Degree of deacetylation
ND: Not detectable

Discussion

Moisture Content

The composition of shell waste, chitin and chitosan obtained from three different sources is given in in Table 1, 2 & 3. Hossain and Iqbal¹⁵ quantified the moisture of shrimp shell waste as 69.3 %. But this study showed that the moisture content of shrimp, crab and squilla were, 21.51%, 20.57 % and 22.59 % respectively. The variation in moisture content may be due to the season, climate, weather and raw material condition¹⁶. The lower result of moisture content of the raw material was mainly due to the collection procedure. Here the raw materials collected were used after straining the liquid. The moisture content of chitin obtained from shrimp, crab and squilla were 8.3, 9.32 and to 9.10 % respectively. These results had shown lower than the moisture content chitin obtained by Abdulkarim *et al.* in mussel shell (12.90%)¹, the reason may be due to the drying conditions of chitin, where the experimental chitin was dried in the hot air oven. Hygroscopic nature of chitosan leads to absorb moisture content from atmosphere when it exposed to outside. According to Sukumaran *et al.*,²⁷ commercial chitosan products should contain less than 10 % moisture. The result of final moisture content of shrimp, crab and squilla chitosan were within the limit viz. 7.56 %, 7.62 and 7.67 %. These results of the study are evident to prove the moisture content of the chitosan is within the standard.

Protein and Lipid Content

No and Lee²⁰ reported that crude protein content of shell fish waste is (7.06 % to 7.97 %) on dry matter. It was found that the crude proteins of the selected shell waste were ranged from 30 to 33 % on wet weight basis. Deproteinisation could not remove 100 % protein from the shell waste. The residues of amino group may express as protein²⁵. Protein is bound by covalent bonds forming stable complex with chitin and chitosan. Even after deprotenisation, the chitin had trace amount (0.8%) of protein. It may be due to the amino (-NH₂) group of the chitosan²⁰. The crude lipid of the shell wastes was around 10 % that describes the relation with crude protein content of the shells. Crude lipid was found neither in chitin nor in chitosan. These results were comparable with 9 % crude lipid in shrimp shell waste by Trung and Phuong²⁹. Brine and Austin¹⁰ also stated about the lower solubility in relation with incomplete removal

of protein. Marwa *et al.*,¹⁷ found the maximum level of deproteinization was around 85 % and 91% for *P. segnis* and *P. kerathurus* shells, respectively.

Ash Content

The level of ash indicates the efficiency of the demineralization (DM) step for removal of minerals. The ash content of the shells was more than 20 % initial stage. It has reduced to 0.48, 0.96 and 0.76 after demineralisation step. The mineral content of crab shells showed more than other two shells because of hard structure due to strong bond between chitin and minerals. The ash content of demineralised crab shell was higher than that of shrimp and squilla. It elucidate that the acid concentration and duration for demineralisation is not sufficient for crab shell waste. Because the crab shells have more mineral contents than the other two shells. Chitosan with less than 1% ash content considered as a high quality grade²⁰. In the present study, the shrimp chitosan showed 0.36 % considered to be the high quality chitosan followed by squilla (0.58 %) and crab (0.76 %). The ash content of crab shell was less than 1% has been reported by No and Meyers²⁰. The residual ash content is considered as an important parameter affects the solubility, consequently reducing the viscosity¹⁵.

Degree of Deacetylation

No and Meyers²⁰ shown the range of degree of deacetylation (DD) of chitosan from 56 % to 99 % with an average of 80 % considered as high quality chitosan. The shrimp chitosan showed superior quality with DD value 71.58 % than squilla (65.54 %) and crab (63.53 %). The lower DD values of chitosan obtained from crab and squilla was agreed with the findings of No and Meyers²⁰ and Toan²⁸ respectively.

Viscosity

The viscosity range of 60 to 5,110 cPs reported by various researcher, which is depends on the source of chitosan^{2,4,8}. These ranges of viscosity have also been supported by the Cho *et al.*,¹¹ with five commercially available chitosans. Bough *et al.*,⁸ reported the viscosity of shrimp and krill chitosan as 5,110 cPs and 5,074 cPs, respectively. It is agreed with the experimental shrimp chitosan as 5300 cPs. The viscosity of shrimp chitosan is of superior quality than squilla (2600 cPs) followed by crab (465 cPs).

The presence of residual ash content and lower molecular weight of the chitosan were decreasing the viscosity of the crab chitosan²¹. Sometime, the deacetylation process with concentrated sodium hydroxide (50 %) usually at 100 °C for 30 min is a harsh treatment. It may revert the demineralization (DM) and deproteinization (DP) process while production of chitosan²⁸. The parameters such as concentration, molecular weight, degree of deacetylation, temperature, pH, Ionic strength are affecting viscosity of chitosan⁸. Moorjani *et al.*,¹⁸ stated that frequent bleaching steps significantly reduces the viscosity of the chitosan so it is not advisable to bleach the final product.

Solubility

The presence of residual ash content reduces the solubility of the chitosan; subsequently affect the viscosity¹⁵. The shrimp chitosan showed 97 % solubility in 1% acetic acid with good viscosity of 5300 cPs. The squilla chitosan showed only 89 % solubility with viscosity of 2600 cps. The poor solubility and viscosity of crab chitosan related with high ash content. The results were agreed with Toan²⁸ experiment on shrimp chitosan.

Yield

The yield of shell wastes from the animal were around 50 % on wet weight basis, but the chitin yield was 17 % for shrimp, 14.5 % for crab and 13 % for

squilla. These results were concurred with shrimp shell chitin estimated by Trung and Phuong²⁹. The chitosan yield from the shrimp showed better result of 15.40 % after reduction of 2 % yield from chitin by the deacetylation process. The shrimp shells yielded significantly more chitosan. David *et. al.*,¹² explained the economic comparison of the yield of chitin and chitosan by experimental lab-scale and commercial plant process. The result showed the same yield for these two processes.

Conclusions

Shrimp, crab and squilla shell wastes were treated with Toan²⁸ chemical extraction procedure to produce chitin and chitosan. As per the research output of the present study, the chitosan obtained from shrimp shell waste appeared to be the highest degree of deacetylation, solubility and viscosity along with yield. Hence it can be concluded that shrimp chitosan appeared of superior quality than crab and squilla chitosan. Utilisation of shrimp shell waste for the production of chitin and chitosan will give more economical and biological value along with reduction of environmental pollution.

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