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Research Article
Extraction and Worth Evaluation of Chitosan from Shrimp and Prawn Co-products

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Abstract

Background and Objective: Shrimp and prawn industries generate a huge amount of co-products (CPs) that can be utilized as a key source of chitin and chitosan, natural multifunctional polymers. The current study modified the existing extraction methods to produce chitin and chitosan from shrimp and prawn co-product (shell). **Materials and Methods:** Two improved methods (M_1 and M_2) with sub-sets (T_A, T_B, T_C) were executed through chemical processes comprising demineralization, deproteinization and deacetylation maintaining different conditions. Chitin and chitosan were extracted by using different concentrations of HCl (1, 1.25 and 1.5 M) in the demineralization step. The purity of chitosan was tested by the ash content, moisture content, solubility test and biuret test. **Results:** Among the sub sets M_2 T_B and M_2 T_A produced higher amounts of chitosan from shrimp and prawn shell, respectively. The yield of the chitin and chitosan were higher in M_2 than M_1 for both species. M_2 method found almost two times faster in time and comparatively pure and commercially standard than M_1. The improvised method M_2 seems to time sparing and efficient than the existing methods. **Conclusion:** Productions of chitosan from co-products will reduce the dependency on import for chitosan and may create employment and, exporting opportunities.

Key words: Co-products, shrimp, prawn, chitin, chitosan, exporting opportunities

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fish and shellfish provide a rich source of high-quality proteins containing all essential amino acids, minerals and micronutrients such as iron, zinc, n-3 long chain polyunsaturated fatty acids (n-3 PUFA) and vitamins, often in highly bioavailable forms[1]. The aquaculture sector in Bangladesh is responsible for over half of all fish production in the country and contributes significantly to national GDP (Gross Domestic Production). Aquaculture seen as one of the most economically and socially important sector in the country as it contributes to food security, livelihoods and the export earnings annually[2]. The total production of fish is about 4.28 million MT in which shrimp and prawn contributes 5.78%[3]. About 32174.7 MT shrimp and prawn is exported to international markets which generates 387.1 million US$ after series of processing to form wide varieties of products such as head on shell on (HOSO), headless shell-on (HLSO), peeled and deveined (P and D), peeled and un-deveined (PUD)[3]. These types of processing also produce co-products (CPs) such as carapace, body shell and claws. It is believed CPs can be a source of chitin (8-10%) and the chitin derivative chitosan[4]. Chitin $(C_8H_{13}O_5N)_n$, a cellulose-like polysaccharide, is the second most abundant biopolymer on earth having an acetamide[5-7] group ($NH\text{-}CO\text{-}CH_3$) at C-2. Chitosan, a linear polysaccharide, contains deacetylated units (D-glucosamine) and acetylated units (N-acetyl-D-glucosamine) which is linked by β (1,4) glycosidic bonds[8]. Chitin and chitosan have many commercial aspects because of their high nitrogen content (6.89%) as compared to synthetically substitute cellulose (1.25%)[9]. Chitin and chitosan have many applications in agriculture, tissue engineering, pharmaceutical industry, water treatment, cosmetics, anti-tumor agent, anti-microbial agent, carriers for active ingredients[10].

Every year around 30000 MT, in wet basis, of shrimp and prawn CPs are previously dumped by the shrimp processing industries of Bangladesh[11]. On the other hand, every year Bangladesh has to import a huge amount of chitosan for its growing food, paper and medicine industries. By 2022, overall global supply of Chitosan will be only 70000 MT against a demand for 155500 MT[12]. There are different methods to extract chitosan from shrimp and prawn CPs that are varying in terms of chemical use, efficacy, timing and so on. Every method has different strengths and weaknesses therefore, to find a time efficient way to extract chitin and chitosan, this study attempted to develop an improvised technique that would facilitate to gain good yield from CPs in local context.

MATERIALS AND METHODS

Study area: The study was carried at the laboratory of Department of Fisheries and Marine Science and Department of Applied Chemistry and Chemical Engineering at Noakhali Science and Technology University, Noakhali, Bangladesh. The work was started in January, 2019 and ended in November, 2019.

Sample collection: Whole black tiger shrimp (*Penaeus monodon*) and giant freshwater prawn (*Macrobrachium rosenbergii*) were collected from the main hotspot of shellfish farming area, Khulna province of Bangladesh. The samples were used to determine the CPs from each type of species. Major shrimp-prawn processing plants and depot are also located in Khulna. Skilled women workers were selected who usually cleaned shrimp and prawn in depot and industry for the cleaning process. Internal validation among the women was calibrated by using same size shrimp and prawn for processing.

Research procedure: Shrimp-prawn carapace, body shell, claw were the major CPs derived from this process. After the cleaning process CPs were then transported to the laboratory maintaining low temperature by using ice (CPs: ice ratio::1:3) within 1 h of cleaning in insulated ice box. In the laboratory, CPs (carapace, body shell, claw) were washed under running tap water to remove soluble organics, adherent proteins and other impurities. Afterward, shells were boiled in water (water: shell:3:1) for 1 h to remove the tissue and move them into an oven at 160°C for 2 h to make them more brittle and then cut into small pieces and pulverized by a grinder. Then each items of shell were weighed, packed in airtight polythene pouch and frozen at -20°C separately. Sodium hydroxide, (E-Merck, Germany), hydrochloric acid (E-Merck, Germany), acetic acid (CH_3COOH) (E-Merck, Germany), De-ionized water and Copper (II) sulphate (E-Merck, Germany), were used for extraction. Extraction of the chitin and chitosan from CPs by chemical treatment method with some modifications from Ahing and Wid[13] and Black and Schwartz[14] was done. All the chemicals were used without any further purification. After these preliminary steps two different types of method (M_1 and M_2) with three subsets (T_A, T_B, T_C) were used to extract chitosan from shrimp and prawn shells.

Method-1 (M_1)

De-mineralization: Dilute hydrochloric acid (1/1.25 and 1.5 M) was used for removing calcium carbonate the main inorganic

part of the shells, to prevent hydrolysis of chitin. The reaction time was varied from 90-120 min. The ratio of dried shells to acid solution used during the extraction of chitin was 1:30 (w:v). The experiments were carried out at room temperature under constant stirring of 150 rpm. The decalcified shells were collected onWhatman No.1 ash less filter paper, washed to neutral condition with de-ionized water and then oven-dried at 70°C overnight. Then the weight of dried sample was measured on electric balance. The rate of demineralization was evaluated by determining ash contents in the solid.

De-proteinization: Sodium hydroxide (1.5 N) concentration was used for 60 min at room temperature to demineralize dried shells and the material was then filtrated using Whatman No. 1 ash less filter paper, washed and dried. The weight of the dried sample and protein concentration in the supernatant was determined according to Biuret's method[15], after this process chitin was found.

De-acetylation: The conversion of chitin to chitosan involved deacetylation process. These parameters (reaction duration, temperature and concentration ofalkaline reagent) were treated as follows: a suspension of 1 g of chitin in 50 mL of aqueous sodium hydroxide as deacetylation reagent (50% by weight) was mixed at room temperature under constant stirring at 150 rpm. After 90 min, the solid was filtrated by 250 micrometer sieve, washed with de-ionized water until the filtrate was neutral. Then it was oven-dried at 70°C overnight and pulverized by grinder as chitosan.

Method-2 (M_2)

De-mineralization: Three samples of 2 g dried shell (shrimp and prawn) were prepared, each with 50 mL of HCl at differing concentrations (1M, 1.25M and 1.5M).Then the sample was heated (60°C) in a water bath for 60-90 min, under constant stirring of 150 rpm. Shells were then filtered by Whatman No. 1 ash less filter paper (pre-dried and weighed) and precipitates were rinsed with boiled de-ionized water for multiple times.

De-proteinization: Placed precipitates and filtrated in a beaker ensuring complete transfer by washing with 1.25 N NaOH and then 100 mL of 1.25 N NaOH was added. Then the sample was heated (60) in a water bath for 60-90 min, under constant stirring of 150 rpm. The sample was filtered by Whatman No. 1 ash less filter paper and washed with boiled de-ionized water 5 times. The sample was placed into an oven at 130°C for 6 h and cooled at desiccator and weighed as chitin.

Deacetylation: Aqueous NaOH, (50% by weight) was added at the ratio of 1:50 (w:v) and heated (60) in a water bath for 60-90 min under constant stirring of 150 rpm. Then filtrated was washed with boiled de-ionized water five times and placed into an oven at 110°C for 6 h. Then the samples were cooled in desiccator and weighed as chitosan.

Chemical analysis of shrimp and prawn shell wastes: Ash and moisture content of shrimp and prawn CP (shell) was determined according to AOAC[16]. To test solubility, 1 g of chitin and chitosan were weighed and dissolved in 100 mL of 1% acetic acid solution. The mixture was stirred well and kept for 2 h 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 percent solubility was calculated from the ratio of weight gain of filter paper x100. The Biuret test was used to determine the presence of protein content in the sample. For Biuret test, chitosan was treated with an equal volume of 1% strong base (sodium or potassium hydroxide) followed by 2-3 mL of aqueous copper (II) sulphate. If there was any protein content in the sample, the color of solution turned into purple[17,18].

Statistical analysis: Data collected from the research was entered and analyzed statistically using the Statistical Package for the Social Sciences (SPSS Inc. version 20.0). One-way ANOVA (Duncan multiple range test, DMRT) was done to know the difference between two methods at 95% significance level.

RESULTS

Quantification of shrimp and prawn describes the percentage of the different portions of body on wet basis. The total body of shrimp and prawn was divided into 2 portions, edible portions (flesh) and CPs (head, carapace, claw, body shell). CPs was 49.34 and 62.65% in shrimp and prawn, respectively in which head had highest amount followed by body shell, claw and carapace (Table 1).

The extraction methods followed the three key stages including demineralization, deproteinization and deacetylation (Fig. 1). In each method there was a sub-set of treatments named T_A, T_B and T_C. Three samples (T_A, T_B and T_C) were treated with 1M, 1.25 M and 1.5 M concentration of hydrochloric acid solution (M_1 and M_2) in the demineralization process, respectively. Then in the deproteinization process, sodium hydroxide solution (1.5 N for M_1 and 1.25 N for M_2) was

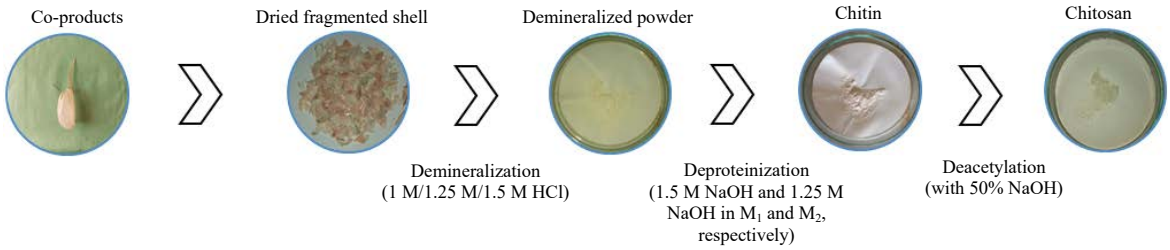

Fig. 1: Steps for extraction of chitin and chitosan from shrimp and prawn shell

Table 1: Quantification of shrimp and prawn (wet basis) body based on different portion

	Wet weight (%) to total body weight	
Body portion in weight (g)	Shrimp	Prawn
Head	32.04±3.64	36.21±2.52
Carapace	3.80±0.95	4.19±1.03
Claw	-	6.05±2.46
Body shell	13.50±3.39	16.20±2.95
CPs (a)	49.34	62.65
Flesh	50.66±3.38	37.35±2.88
Edible portion (b)	50.66	37.35
Total weight (a+b)	100.00	100.00

Table 2: Comparison of yield from shrimp by deproteinization (1.5 N NaOH and 1.25 N NaOH respectively in M_1 and M_2) and deacetylation (50% NaOH in both M_1 and M_2)

	Chitin (%)		Chitosan (%)		Time (h)		Color of chitosan	
Treatments (HCl concentration, M)	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
T_A (1)	23.43±0.21^c	25.70±0.30^a	21.44±0.19^c	23.24±0.24^a	36.02±0.38^a	16.64±0.14^d	White	White
T_B (1.25)	22.70±0.20^d	25.33±0.20^a	20.75±0.12^d	22.97±0.15^a	34.13±0.97^b	15.17±0.61^e	Brilliant white	Brilliant white
T_C (1.5)	22.13±0.15^e	24.37±0.30^b	20.07±0.15^e	22.40±0.26^b	31.19±1.14^c	14.78±0.83^e	Super white	Super white

Different superscripts in each box (chitosan/chitin/time) indicating the differences significantly ($p<0.05$)

Table 3: Comparison of yield from prawn by deproteinization (1.5 N NaOH and 1.25 N NaOH respectively in $M_1$and M_2) and deacetylation (50% NaOH in both M_1 and M_2)

	Chitin (%)		Chitosan (%)		Time (h)		Color of chitosan	
Treatments (HCl concentration, M)	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
T_A (1)	23.56±0.19^c	26.40±0.26^a	21.50±0.26^c	23.63±0.15^a	35.08±0.29^a	15.49±0.21^c	White	White
T_B (1.25)	22.57±0.25^d	26.33±0.25^a	20.60±0.20^d	22.67±0.21^b	34.25±0.86^{ab}	14.37±0.75^c	Brownish white	Super white
T_C (1.5)	21.57±0.31^e	25.37±0.25^b	20.50±0.30^d	22.43±0.15^b	33.12±1.19^b	14.09±0.92^c	Super white	Super white

Different superscripts in each box (chitosan/chitin/time) indicating the differences significantly ($p<0.05$)

used. In the deacetylation process, the final step of the preparation of chitosan, 50% sodium hydroxide solution was used. Chitin production was higher in M_2T_B (25.33±0.20%) in shrimp and M_2 T_A (26.4±0.26%) in prawn, respectively (Table 2 and 3). Production of chitosan was higher in M_2T_A (23.24±0.24 and 23.63±0.15% in shrimp and prawn, respectively) (Table 2 and 3) than all other sub-sets of experiment. The time required for the second method (M_2) was almost half of the first method (M_1) (Table 2 and 3). The more rapid production of chitosan as a result from, the M_2 method may be a more cost-effective way to produce this product. The color of chitosan was better in quality in T_B (brilliant white) and T_C (Super white) than T_A (white) from both methods (Table 2 and 3).

The production of chitin and chitosan can be different due to different species, different methods, different parameters used or conditions during the deacetylation process (Table 4).

The moisture content was 1.28±0.22 and 1.27±0.18% (M_1) as well as 1.25±0.09 and 1.26±0.14% (M_2) in shrimp and prawn, respectively. The ash content was found 1.26±0.17% and 1.20±0.10% (shrimp) and 1.25±0.11 and 1.21±0.07% (prawn), respectively in M_1 and M_2 (Table 5). Solubility test is one of the most important factors to determine the quality of

Table 4: Comparison of yield of chitin and chitosan with previous studies

Regions	Species	Chitin (%)	Chitosan (%)	Color	References
Bengaluru, India	Shrimp	14.72±0.57	12.03±0.46	-	Naznin[18]
Khulna, Bangladesh	*Metapenaeus monoceros*	22.60	21.76	White	Abdulkarim *et al.*[19]
Nigeria	Mussel	23.25	15.14	-	Alishahi *et al.*[20]
North Iran	*Metapenaeus monodon*	24.33±0.19	15.25±0.90	-	Aranaz *et al.*[21]
Bangladesh	*Penaeus monodon*	25.33±0.20	22.97±0.15	Brilliant white	Present study (T_BM_2)
	Macrobrachium rosenbergii	26.40±0.26	23.63±0.15	White	Present study (T_AM_2)

Table 5: Characterization of chitosan deriving from shrimp and prawn shell

	Moisture (%)		Ash (%)		Solubility test (%)		Biuret test	
Raw sample	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2
Shrimp	1.28±0.22	1.25±0.09	1.26±0.17	1.20±0.10	99.0±2.98	99.5±1.19	Color unchanged	Color unchanged
Prawn	1.27±0.18	1.26±0.14	1.25±0.11	1.21±0.07	99.4±2.84	99.6±1.23	Color unchanged	Color unchanged

chitosan, where higher solubility means better chitosan produced. The color of chitosan (M_1 and M_2) was unchanged after biuret test which indicate the absence of protein (Table 5).

The total production of shrimp and prawn was 61709 MT and 51571 MT in the year of 2017-18, respectively. According to this study, about 49.34 and 62.65% co-products are produced from shrimp and prawn, respectively. From these CPs, raw materials for chitosan contains 17.30% in shrimp and 26.44% in prawn. About 10675.66 MT and 13635.37 MT co-products (carapace, claw and shell) could be produced from shrimp and prawn, respectively. About 2452.2 MT (M_2T_B) and 3222.1 MT(M_2T_A) chitosan could be found from shrimp and prawn which is worth of 245.2 million US$ and 322.2 million US$, separately (1 kg/100 US$). Therefore, by converting these co-products into value, Bangladesh can reduce the present dependency on import for chitin and chitosan which may open a new door for exporting it to the other countries as well.

DISCUSSION

Abdulkarim *et al.*[19] reported 15% yield of chitosan from shrimp shell waste which is slightly lower than yield of chitosan in the present study. The variation could be due to difference in the methodology of chitosan production and age of the shrimp and prawn from which the sample was taken[20]. The experimental results show that M_2 yields better chitin and chitosan content as compared to the M_1. The main difference between the two methods is heating the CPs during deproteinization step in case of M_2. Usually, shrimp and prawn contain 25-40% protein of which 60-75% of this consists of collagen and the rest consists of elastin and keratin protein[21]. Literature study showed that the removal of both collagenous and non-collagenous protein is accelerated by the addition of alkali like NaOH[22]. However, increase in temperature can also increase the solubility of collagen in basic medium[23]. As a combination of alkali, heat and stirring effect was applied in M2, it results in a better removal of protein and subsequently better yields of chitin and chitosan as compared to the M1 method. Moisture content for chitosan in shrimp and prawn was much lower than Alishahi *et al.*[20] in North Iran. Moisture content affects the quality of chitosan produced from co-products and Szymanska and Winnicka[24] also suggested that the moisture content of chitosan must be low to prevent damage of the polymer. Islam *et al.*[2] reported that the ash content was 1.5% on dry wet basis in shrimp shell which is similar to present study. The solubility rate was 99±2.98 and 99.4±2.84 (M_1) then 99.5±1.19 and 99.6±1.23% (M_2) in shrimp and prawn, respectively which is similar to Ahing and Wid[13] at Sabah (Table 5). The color of chitosan produced from the present study is similar to the chitosan obtained from Naznin[18]. Therefore, as a cost effective and an efficient technique, it will be eventually help the entrepreneurs to adopt this technology in Bangladesh and wider afield.

CONCLUSION

The shellfish (shrimp and prawn) processing industry is rapidly growing in Bangladesh and around the world. A vast amount of co-products produced from these processing industries can be a vital source of many useful substances including chitin and chitosan. In this present study, co-products from 2 different species (*P. monodon, M. rosenbergii*) were used to obtain chitin and chitosan by improvised techniques (M_2) which are more convenient

than previous methods (M_1) according to the handling procedure, time, yield and the accuracy of chitosan production.

SIGNIFICANCE STATEMENT

This improvised technique will help to create local entrepreneurship for extracting chitosan from shrimp and prawn co-products. The co-products have the potential to be used in producing a good quality chitosan that can be applied in fields such as agriculture, food industry, pharmaceuticals, textiles, wastewater treatment, cosmetics and which may also improve the economy improve the economy by creating employment opportunities.

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