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Production of chitin and chitosan from shrimp shell wastes

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Abstract

A method was developed for commercial scale production of chitin and chitosan in Bangladesh from marine shrimp, *P. monodon* and freshwater prawn, *M. rosenbergii* shell and appendages. Chitin is a macro-molecular linear polymer of anhydro N-acetyl glucosamine (N-Acetyl, 2-Amino 2-Deoxy D-Glucose) and chitosan is deacetylated chitin. For production of chitin, fresh shells of *P. monodon* having initial bacterial load of $>10^5$ CFU/g sample and peroxide values of >10 mmol free iodine liberated /kg of oil were washed with dilute sulfuric acid. Adhered proteins were removed by washing with low strength alkaline solution and then rinsed with water. Crude chitin thus prepared was treated with concentrated hydrochloric acid and purified chitin was obtained after treating with low strength alkali solution. Water soluble chitosan was prepared by performing a deacetylation process using 50% NaOH (w/w) at 100^{o}C for 4-5 hours and then washed, dried and ground. For purification of chitin and chitosan, a series of experiments were conducted to optimize the level of NaOH concentration and time and temperature schedule of demineralization and deproteinization/deacetylation. A high temperature-short time schedule obtained best quality chitin and chitosan. Both subjective and objective methods were used for the testing of quality and purity of chitin and chitosan. Comparative studies between the quality of products from different components of the shell and from different shrimp/prawn species showed that both chitin and chitosan obtained from *M. rosenbergii* shell were better compared to those of *P. monodon* in terms of extractability, deacetylation, and color. Shells obtained better product compared to shrimp appendages. The study suggests that chitin and chitosan can be produced in existing shrimp/prawn processing plants of the country with the simple renovation.

Keywords: Shrimp, Shrimp industry wastes (shrimp shell, head, appendages), Chitin and chitosan

Introduction

Fisheries sector plays an important role in the nutrition, socio-economic development and poverty alleviation of a large number of population of Bangladesh. The contribution of the sector to gross domestic product, foreign exchange earnings, and employment is also significant. In Bangladesh, shrimp and prawn are considered as very important aquaculture products due to its popularity. In the year 2006, Bangladesh earn US $456 by exporting shrimp (BFFEA, 2008) and Bangladesh ranked 6th in case of volume of production (Rahman MM and Hossain MM, 2009). With the production of exportable frozen products, a huge quantity of wastes (around 40-80%, depending upon species and process) are produced by processing plants. (Suparno and Nurchaya, 1984; Suparno and Poernomo, 1992; Irianto and Giyatmi, 1997). The solid waste contains mainly head, shell, tail and vein/viscera (Khan and Nowsad 2013). In the processing plants in Bangladesh, shrimp wastes are generally treated as trash and for the disposal of wastes either extra money is spent or additional manpower is needed (Nowsad, 2005). In the form of head, shell, leg, appendages and tail, around 40-50% of the shrimp is wasted and according to an estimate, every year 30,000 tons of shrimp waste are dumped by shrimp processing industries of Bangladesh (Nowsad, 2005).

Shrimp shell contains a huge amount of chitin (8-10%) which is an expensive ingredient used in many foods, cosmetics and pharmaceutical products (Suparno and Poernomo, 1992). Bangladesh has been importing about 100-120 tons of chitin and chitosan annually, mainly for food and medicine industries (BBS, 2005). Production of chitin within the country can reduce the present dependency on import for this valuable raw material. If a nominal care is taken, it is possible to produce both chitin and chitosan within the existing process line of the shrimp processing plants. The products can either be marketed locally or exported. Therefore this research aimed at developing appropriate field supported techniques for the commercial manufacture of chitin and chitosan from shrimp shell wastes.

Chitin is a high molecular weight linear polymer of N-acetyl-D-glucosamine (N-acetyl-2-amino-2-deoxy-D-gluco-pyranose) units linked by beta-D (1- 4) bonds. It is a highly insoluble material. It may be regarded as cellulose with the hydroxyl at position C-2 replaced by an acetamido group. Like cellulose, it naturally works as a structural polysaccharide. It is most abundant in crustaceans, insects, and fungi. Chitin is a hard, inelastic, white in color, nitrogenous polysaccharide and Chitosan is the N-deacetylated derivate of chitin. Chitin and chitosan are of commercial interest because of their high percent of nitrogen (6.89%) compared to synthetically substitute cellulose (1.25%) (Gupta and RAVI KUMAR, 2000) and this makes chitin a useful chelating agent. Many reviews and articles have been published covering the applications of chitin and its derivatives in the area of pharmaceutical, biomedical applications, paper productions, textile finishes, photographic products, cement, heavy metal chelating agents, cosmetics, effluent treatment methods and engineering applications, for example, solid state batteries (Dutta and Tripathi, 2004). It was, therefore, felt necessary to determine a low-cost chemical technique which would be suitable for the production of chitin and chitosan from shrimp shell wastes. This new production technique, if determined, would open the avenue of serving six important purposes: i) Low-cost suitable techniques for chitin and chitosan production from shrimp wastes determined ii) New item included in the export list that can earn foreign currency accounting several hundred crores taka iii) Import of these materials reduced iv) Unutilized or underutilized shrimp wastes properly utilized instead of dumping and saved several crores of Taka required for disposing those wastes v) Pollution free safe environment and vi) Eco-friendly, cost-effective waste management practice established in the plants.

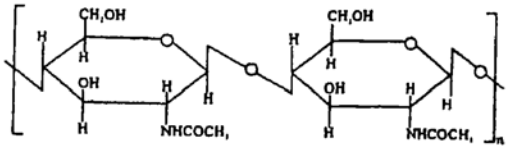

Fig. 1. chemical structure of chitin

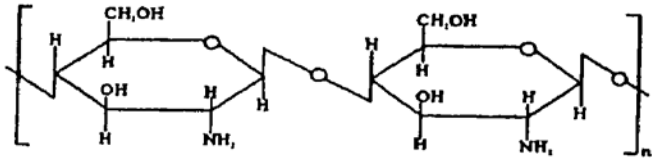

Fig. 2. chemical structure of chitosan

Materials and Methods

Laboratories of the Department of Fisheries Technology, Bangladesh Agricultural University (BAU), Mymensingh was the place, where this experiment was performed. Sources of shrimp wastes were Lokpur Seafood Ltd. processing plant, situated in Khulna and transported to the laboratory of BAU maintaining low temperature (properly iced shrimp wastes within insulated ice box). After brought to the laboratory, attached meats / shrimp flesh were removed from the shrimp wastes and washed thoroughly. They were either crushed and packed or directly packed in airtight polythene pouch and then frozen stored in a deep freezer at -20^0 C.

Chemical analysis of shrimp shell (*P. monodon*) wastes

Proximate composition of shrimp shell wastes was determined as moisture (Ludorff and Meyer, 1973), crude ash (AOAC, 1990), crude protein (AOAC, 1990) and crude fat (Bligh and Dyer, 1959). The pH was determined by using a digital pH meter at room temperature. Three replications of analysis were done. Determination of peroxide value was also done (AOAC, 1990).

Preparation of chitin and chitosan

Chitin and chitosan were produced in two steps. First, production of chitin from shrimp wastes and then production of chitosan from the prepared chitin.

Production of chitin from shrimp shell

The fresh shell of shrimp or prawn were washed, weighed and taken into a beaker. Four volumes of 1.25 N HCl was added to the beaker. After 3 hours the acid-mix sample was washed with water. Again 4 volumes of 1.25 N HCl was added and kept it for overnight. The sample was washed with tap water and dried for few minutes. In this way, the remaining minerals were eliminated from the shell. Then it was

deproteinized by adding 5 volumes 5% NaOH (w/w) and then heated in the water bath for 1 hour at 70-75 ^{0} C. To remove last protein portion it was deproteinized again and again by the same method. After deproteinization, the sample was again washed with tap water carefully and dried in an oven at 65^{0}C for 8 hrs. The material was then pulverized as chitin. Scheme for the preparation of chitin from *P. monodon* shell is shown in Fig. 3.

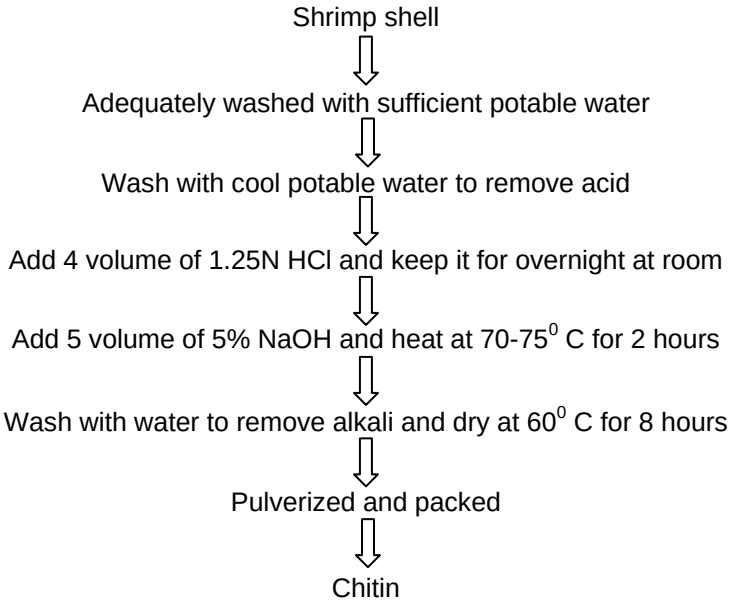

Fig. 3. Scheme for the production of chitin from shrimp shell

Production of chitosan from chitin

To produce chitosan, the produced chitin was washed and dried for few minutes. Then 5 volumes of 40% NaOH was added and heated at 100^0 C for 5-6 hours. NaOH was drained; the product was washed with cool potable water for several times and dried in the oven at 65^0 C for 8 hours. Finally, the product was pulverized and packed as chitosan. Scheme for the development of chitosan from chitin (Shrimp shell *P. mondon*) is presented in Fig. 4.

Standardization and purification of the production process and products:

Standardization of the process and purification of the products were done by trial and error method of setting up production parameters like pre-washing with different concentration of low acids, extraction with increased concentration of NaOH, changing with fresh NaOH solution for several times and extraction of different high temperatures.

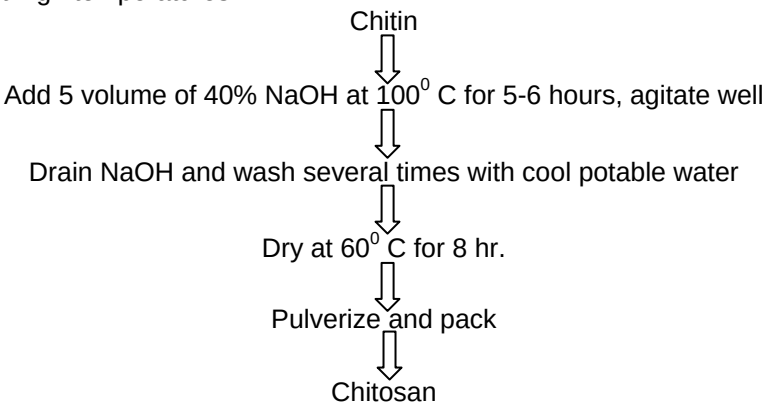

Fig. 4. Scheme for the production of chitosan from chitin (shrimp shell)

Quality analysis of chitin and chitosan

Both subjective and objective methods were used to determine the quality and purity of chitin and chitosan for 12 months. Subjective methods included sensory analysis, whiteness, pulverizing property, etc., and objective methods included biochemical parameters, moisture content, reabsorption property and solubility by instrument. Sensory analysis was done by color and pulverizing property. The color of the products were determined by giving scores of 0-5, with 0 being the reddish color and 5 being the bright-white color. AOAC (1990) methods were followed for estimation proximate composition. For each analysis of proximate composition, triplicate samples were used. Product pH was measured as before.

For solubility test, weighed chitin and chitosan (1g) were dissolved in 100 ml of 1% acetic acid solution. The mixture was stirred well and kept at ambient temperature for two hours. The solution mixture was passed through a pre-weighed Whatman No. 1 filter paper. After passing out of all the solvent, the filter paper was dried at ambient temperature and re-weighed. The percent solubility was calculated from the ratio of weight gain of filter paper x100. If insoluble, it would remain with filter paper and weight increased, but if soluble, nothing will remain with filter paper and therefore, the weight of filter paper would not change. Reabsorption ability of the products were measured in percent by keeping chitin and chitosan in air at ambient temperature. A known quantity (5g) of chitin and chitosan was kept open in the crucible and reabsorption ability (%) was calculated from the ratio of weight gain × 100.

Results

Proximate composition

Each part of shrimp shell waste components contained very good nutritional contents (Fig. 5).

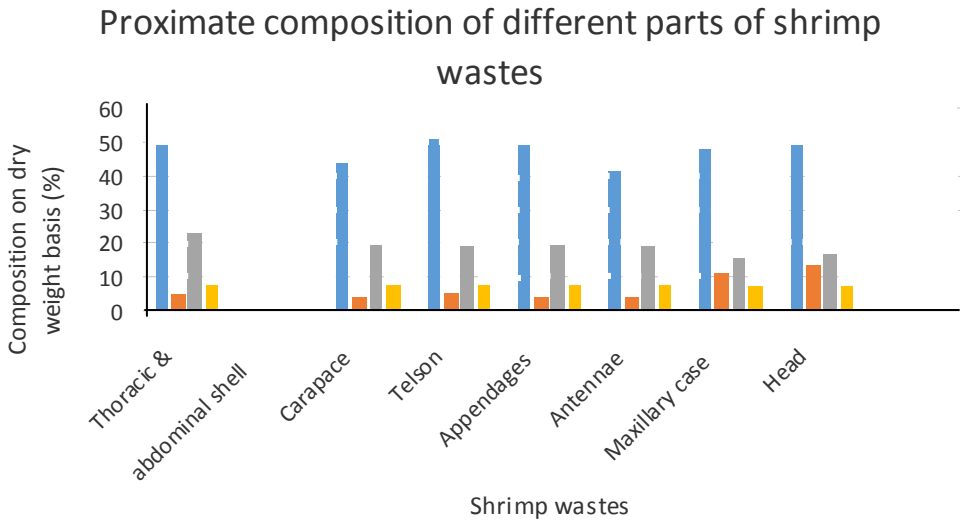

Fig. 5. Proximate composition of different parts of shrimp wastes

As of common method, for the production of crude chitin, shrimp/prawn shell pastes were washed with dilute sulphuric acid. Adhered proteins were removed by washing with a weak alkaline solution and then rinsed with water. Demineralized shells were pulverized and crude chitin was obtained. Crude chitin was treated again with concentrated hydrochloric acid and purified chitin was obtained as a precipitate. Water soluble chitosan was prepared by performing a deacetylation process using concentrated NaOH (w/w) at

70-80^{o}C for 2 hours. Finally, chitosan was washed, dried and ground. Production methods of chitin and chitosan were refined, fine-tuned and field validated. For production of chitin, fresh shells of *Penaeus monodon* and *Macrobrachium rosenbergii* having initial bacterial load of <10^5 CFU/g sample and peroxide values of <10 m mol free iodine liberated /kg of oil (Table 1) were washed with 0.2 – 0.3% dilute sulfuric acid. Adhered proteins were removed by washing with weak NaOH solution and then rinsed with water. For purification, crude chitin was treated with 1.5 N hydrochloric acid and 4-5% alkali solution. From the chitin, chitosan was prepared by treating them with highly concentrated alkali, as much as 50% NaOH (w/w) at 100^{o}C for up to 5 hours.

Table 1. Biochemical qualities of *P. monodon* shell and crude chitin and chitosan

Component	Composition (%) on dry weight basis				pH	Peroxide value (mmol/kg)	APC (CFU/g)
	Moisture	Protein	Lipid	Ash			
Shell	20.5	48.7	4.4	22.5	7.0	9.2	8.4 x 10^4
Chitin	10.9	3.3	0.0	2.8	7.4	-	-
Chitosan	8.2	1.6	0.0	1.5	8.0	-	-

Fig. 1 shows the revised production protocol of chitin and chitosan from shrimp shell wastes. Quality both chitin and chitosan were evaluated through biochemical analysis. Biochemical qualities of chitin and chitosan have been given in Table 2. Prepared chitin and chitosan had protein contents of 3.3% and 1.6% respectively while lipid content was nil. This was further verified as with no peroxide values found for chitin and chitosan samples (Table 1).

Quality of chitin and chitosan prepared from different species and shell components

Chitin and chitosan were prepared from different species of shrimp/prawn and different parts of the shell. The results are presented in Table 2.

Table 2. Quality of chitin and chitosan from the shell of different species

Raw material source	Chitin				Chitosan			
	Extraction time (hr)	Protein (%)	Lipid (%)	Color	Extraction time (hr)	Protein (%)	Lipid (%)	Color
P. monodon shell	3.5±0.5	3.5	2.8	Brown-white	5.0±0.1	1.2	nil	Off-white
M. rosenbergii shell	2.0±0.5	1.8	nil	Brown-white	4.0±0.5	nil	nil	Brilliant off-white
P. monodon carapace	3.0±0.5	2.0	nil	Brown-white	4.5±0.5	1.0	nil	Off-white
M. rosenbergii appendages	2.0±0.5	2.3	nil	Brown-white	4.0±0.5	nil	nil	Off-white

Quality assessment of chitin and chitosan

Moisture, ash and crude protein contents in purified chitin and chitosan were 9.6 and 7.3, 1.7 and 1.0 and 1.8 and 0.0 percent respectively. Lipid was nil in both the products. Colour was developed from brownish white in chitin to bright off-white in chitosan. The pH was 7.3 and 7.8 respectively in chitin and chitosan and reflected well with the solubility data in 1% acetic acid, where chitosan was mostly soluble but chitin did not. Both the products were kept airtight at ambient temperature for 12 months and found in very good quality in terms of solubility, pH and reabsorption ability.

Table 3. Characteristics of purified chitin and chitosan in the laboratory

Attributes	Chitin	Chitosan
Moisture %	9.6	7.3
Ash %	1.7	1.0
Protein %	1.8	0.0
Lipid %	nil	nil
Colour	Brownish white	Off white
Solubility in 1% acetic acid	Not soluble	Soluble (99.5%)
PH	7.3	7.8
Reabsorption ability %	8	9
Particle size	25 mesh	65 mesh
Shelf life	12 months	12 months

Discussion

Shell wastes contain high amount of protein, comprising 40 to 50%. Different shell waste components contain different percentage nutritional contents, such as value of proximate compositions were lowest in antennae and carapace but value is highest in telson part. Shahidi (1994) and Revanker (1978), also found a crude protein content 44 to 52% from shrimp's waste. Although it was considered that antennae contain low nutritional content but were found to have 40.7% protein on dry weight basis. Average protein content of the whole shell wastes was 45.2±1.3%. As shrimp wastes contain extremely high amount of protein, so it widened the scope of utilization and in addition to chitin and chitosan, it can also be used in human food.

Plate 1. Purified chitin and chitosan from shrimp shell wastes

Average lipid content was 4.1±0.5% and head contained highest lipid content, 12.8%. True shell (carapace, appendages, etc) had about 3.4 t0 3.8% lipid. Minerals contents in shrimp wastes were also found to be very high (Fig. 5).

Both the species responded well with the revised extraction methods for the production of chitin and chitosan. This response was, however, swifter in the case of freshwater prawn, *M. rosenbergii*, as extraction time was comparatively less for this species. Chitin was extracted within 2 hours from *M. rosenbergii* shell and appendages, while it took 3 to 3.5 hours for *P. monodon*. 4.5 to 5 hours were required to obtain chitosan from *P. monodon* shell and appendages, while extraction period was 0.5 to 1.0 hour less in the case of *M. rosenbergii*. *M. rosenbergii* obtained better quality products in terms of deproteinization and color too (Table 3). Brilliant off-white chitosan was obtained from this species. This might be due to the initial higher redness of shell of *P. monodon* which became more reddish during heat treatment due to chemical binding of pigments. On the other hand, comparatively whiter shell with less redness during heat treatment assisted in obtaining whiter products from *M. rosenbergii*.

For the purification of chitin and chitosan, a series of experiments were conducted to optimize the level of NaOH concentration and time and temperature schedule of demineralization and deproteinization / deacetylation. A high temperature-short time schedule obtained best quality chitin and chitosan.

Conclusion

A method was developed for commercial scale production of chitin and chitosan in Bangladesh from marine shrimp, *P. monodon* and freshwater prawn, *M. rosenbergii* shell and appendages. Export-oriented shrimp/prawn processing industries in Bangladesh produce about 30,000 tons of waste in the form of a shell, head, viscera, and appendages. We have developed a low-cost technique of commercial production of medicinal products, chitin and chitosan, from these factory wastes. Chitin is a macro-molecular linear polymer of anhydro N-acetyl glucosamine (N-Acetyl, 2-Amino 2-Deoxy D-Glucose) and chitosan is deacetylated chitin. Both chitin and chitosan have very wide industrial application in more than 200 different fields like paper, textiles - sizing, dyeing and printing, chromatography, water purification, effluent treatment, cosmetics, drugs, pharmaceuticals, surgery and many others. Every year Bangladesh imports huge quantity of chitin and chitosan for different industrial use. This new production technique has opened the avenue of serving three important purposes: i.) the country can be able to earn substantial amount of foreign currency by exporting chitin and chitosan or save the currency spent by reducing their imports; ii) instead of disposal, the shrimp processing factory can be able to realize handsome profit by introducing simple process-line for the products; and iii) effective waste recycling can help develop an environment-friendly waste management system to improve plant sanitation and minimize environmental pollution. The study suggests that chitin and chitosan can be produced in existing shrimp/prawn processing plants of the country with the simple renovation.

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