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Extraction of Chitin and Chitosan from Shrimp (*Metapenaeus monoceros*) Shell by Chemical Method

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Abstract: The present study was undertaken to extract chitin and chitosan by chemical method. Several treatments of acid and alkali were taken into consideration to determine effective concentration for yielding optimum output. The shells from Hariana Shrimp (*Metapenaeus monoceros*) were used as raw material in the experiment. Extraction of chitin and chitosan using different concentration of 0.5, 1.0, 1.5 and 2 N commercial grade sodium hydroxide in deproteinization step and different percentages of 10, 20, 30, 40 and 50% commercial grade HCl acid in demineralization step in laboratory scale were done. The dry sample was immersed in HCl solution for demineralization at ambient temperature for over night. Then the sample was collected, washed and dried. To obtain chitosan, deacetilation was carried out by 50% sodium hydroxide solution over night at 60-70°C. In 1% acetic acid solution chitosan dissolved but chitin was insoluble in this solution. Among all treatments, of this research, 30% HCl and 1.5 N NaOH solution yielded better quality chitin and chitosan. The final products were white in color and insoluble in water.

Key words: Chitin, chitosan, deproteinization, demineralization, deacetylation

INTRODUCTION

Shrimp processing industry is rapidly growing concern in Bangladesh and all over the world. A huge amount of shrimp bio-waste is produced from these industries because, shrimps are normally sold as head less and often peeled of the outer shell[1]. This leads an inevitable increase in waste produced by the shrimp industry as 40-45% of the raw shrimps caught are largely of no use[2]. This bio-waste is then considered in landfills, soil dumping and discarded in seawater, resulting the major surface pollution in coastal areas and constituting an important concern of environmental pollution. But the shrimp waste contains the biopolymers, viz., chitin, chitosan, protein and astaxanthin with high economical values. In Southeast Asia, the total waste is produced over 2 million metric tons/year[3]. Production cost for 1 kg of chitosan is about US$ 15-20 kg^{-1} but the price of better quality chitosan (degree of deacetylation is usually in the range of 60-80%) is around US$ 200 kg^{-1} [4]. Twelve kilogram better quality chitosan is obtained from 200 kg shrimp bio-waste[5]. So, it is estimated that only Southest Asia can earn 24000 million US$/year from this bio-waste. It is essential to ensure the full utilization of shrimp waste on a global scale because, processing of these valuable components might offer an economical alternative for any shrimp based country.

Chitin, a nitrogen containing polysaccharide, related chemically to cellulose is a principal constituent of the exoskeleton or outer covering of insects, crustaceans and arachnids. It is high molecular weight linear polymer of N-acetyl D-glucoseamine[6] and insoluble in most of the solvents. Controlled deacetylation to produce derivatives with approximately 50% free amine can be used to produce water soluble chitin[7], called chitosan.

Chitin and its derivatives have many properties that make them attractive for a wide variety of applications, from food, nutrition and cosmetics to biomedicine, agriculture and the environment[8-10]. Their antibacterial, antifungal and antiviral properties make them particularly useful for biomedical applications[11] such as wound dressings, weight loss agent, blood cholesterol control[12], surgical sutures and aid in cataract surgery and periodontal disease treatment[13]. Research has shown that chitin and chitosan are non-toxic and non-allergenic, so the body does not reject these compounds as foreign invaders. Biocompatibility, biodegradability and adsorption properties of chitin and its derivatives are much higher than synthetically substituted cellulose[14].

The objectives of this research were to determine the suitable acid and alkali concentration for extraction of high quality chitin and chitosan from shrimp shell by chemical method and to evaluate the quality and standardization of the final products according to the applied acid base concentration.

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MATERIALS AND METHODS

Chitin and chitosan are produced from shrimp shell through demineralization, deproteinization and deacetylation. This research was carried out by Bioprocess Technology laboratory of Biotechnology and Genetic Engineering Discipline, Khulna University, Khulna, Bangladesh. Shrimp shells were collected from Sigma Sea Foods, a local shrimp processing industry located at Rupsha, Khulna, Bangladesh, during the month of May to July, 2004.

The species was *Metapenaeus monoceros,* local name is hariana. For the chitin and chitosan production each experiment was conducted in three replicates. Completely Randomized Design have been followed in this research. Biochemical composition (protein content, lipid content, ash content and moisture content) were determined to evaluate the quality and standardization of the final product.

RESULTS

Chitin and chitosan production: From these results, it was observed that for chitin and chitosan production, the best acidic (HCl) concentration is 30% and the best alkaline (NaOH) concentration is 1.5 N (Table 1 and 2). At this acidic and alkaline concentration, chitin was produced 9.00±0.0408 g and chitosan was produced 8.7±0.2161 g.

Table 1: Percent distribution of chitin from shrimp species *M. monoceros* shells on dry basis

Raw materials		Acid (HCL) concentration	Base (NaOH) concentration	Weight (g) of chitin			Solubility
Weight (g)	Appearance	(%)	(N)	(Mean±SE)	(%) of chitin	Product appearance	in acetic acid
40	Brown	10	0.5	12.20±0.2274	30.6	Brownish	Not dissolved
			1.0	11.60±0.0817	29.1	Brownish	
			1.5	11.50±0.1081	28.7	Brownish	
			2.0	11.10±0.1472	27.7	Less brownish	
		20	0.5	11.20±0.1225	28.0	Brownish	
			1.0	11.10±0.1472	27.7	Brownish	
			1.5	11.60±0.1780	26.4	Less brownish	
			2.0	10.00±0.1081	24.9	Slightly brownish	
		30	0.5	9.70±0.0204	24.4	Less brownish	
			1.0	9.60±0.0187	24.0	Slightly brownish	
			1.5	9.00±0.0408	22.6	White	
			2.0	8.70±0.1414	21.8	White	
		40	0.5	9.50±0.0817	23.8	Brownish white	
			1.0	9.10±0.0817	22.6	White slightly brownish	
			1.5	8.40±0.2274	21.1	White	
			2.0	8.10±0.1472	20.2	White	
		50	0.5	8.80±0.0817	22.1	White slightly brownish	
			1.0	8.30±0.1081	20.7	White	
			1.5	7.70±0.2274	19.4	Super white	
			2.0	7.20±0.2274	18.2	Super white	

Table 2: Percent distribution of chitosan from shrimp species *M. monoceros* shells on dry basis

Raw materials		HCL concentration	NaOH concentration	Weight (g) of chitosan			Solubility
Weight (g)	Appearance	(%)	(N)	(Mean±SE)	(%) of chitosan	Product appearance	in acetic acid
40	Brown	10	0.5	12.0±0.1414	30.00	Brownish	Slightly dissolved
			1.0	11.2±0.1081	28.00	Brownish	Slightly dissolved
			1.5	10.8±0.0817	27.00	Less brownish	Slightly dissolved
			2.0	10.4±0.3083	25.20	Less brownish	Slightly dissolved
		20	0.5	11.1±0.0817	27.80	Brownish	Slightly dissolved
			1.0	10.8±0.0817	27.50	Less brownish	Slightly dissolved
			1.5	10.3±0.2122	25.80	Slightly brownish	Half of the sample dissolved
			2.0	9.7±0.1225	24.30	White (slightly brownish)	Half of the sample dissolved
		30	0.5	9.6±0.0354	24.10	Less brownish	Half of the sample dissolved
			1.0	9.5±0.0147	23.80	Slightly brownish	More than 0.5 N
			1.5	8.7±0.2161	21.76	White	Almost completely dissolved
			2.0	8.2±0.0817	20.50	White	Almost completely dissolved
		40	0.5	9.1±0.0707	22.80	Slightly brownish	Half of the sample dissolved
			1.0	8.9±0.1081	22.20	White	More than 0.5 N
			1.5	8.2±0.2274	20.60	White	Almost completely dissolved
			2.0	7.8±0.2122	19.50	White	Almost completely dissolved
		50	0.5	8.3±0.1472	20.90	White (slightly brownish)	Half of the sample dissolved
			1.0	7.8±0.1414	19.50	White	Almost completely dissolved
			1.5	7.1±0.1414	17.80	Super white	Almost completely dissolved
			2.0	6.2±0.1472	15.40	Super white	Almost completely dissolved

Protein content: The amount of the protein content of chitosan which were treated with 0.5, 1.0, 1.5 and 2.0 N NaOH concentration are 40.88, 36.50, 32.87 and 31.84%, respectively. Acid concentration was same (30% HCl) for each treatment.

Lipid content: The lipid content (%) of chitosan (treated with same base concentration but 10, 20, 30, 40 and 50% acid concentration) are 4.01, 3.0, 1.78, 0.86 and 0.70%, respectively.

Ash content: The percentage of ash content of chitosan which were treated with 10, 20, 30, 40 and 50% acid concentration and with same base concentration (1.5 N) are 0.38, 0.31, 0.15, 0.08 and 0.06%, respectively.

Moisture content: Moisture content of chitosan which were treated with 10, 20, 30, 40 and 50% acid concentration are 8.01, 7.53, 7.44, 7.31 and 6.62%, respectively. Base concentration was same (1.5 N) for each treatment.

DISCUSSION

On dry weight basis, 40 g of each sample was treated with 10, 20, 30, 40 and 50% commercial grade hydrochloric acid (HCl) to examine the effect of demineralization and for testing the effect of deproteinization, the sample of each acid concentration was treated with 0.5, 1.0, 1.5 and 2.0 N commercial grade NaOH solution.

The results indicate that when acid and base concentrations are increased in demineralization and deproteinization step, respectively, chitin and chitosan production slightly decreases due to extensive demineralization and deproteinization (Table 1 and 2). This experiment was conducted to obtain more demineralized and deproteinized end products which will lead to loss of weight from shrimp shell. The removal of more protein, lipid, pigments and other inorganic acid bring a more white color end product. The end product which contains more chitin, must be whiter in color and the little brownish will be less chitin containing end product and full brownish will be the lowest grade of end product which contain lowest chitin content due to incomplete demineralization and deproteinization. According to color (Fig. 1-4) and weight loss (Table 1 and 2) of the end product, it is possible to identify the chemical (acidic and alkaline) concentration which produces the best chitinous end product.

Fig. 1: Raw material

Fig. 2: Product after deacetylation (10% HCl, 1.5 N NaOH) dry basi

Fig. 3: Product after deacetylation (30% HCl l, 1.5 N NaOH) dry basi

Fig. 4: Product after deacetylation (50% HCl, 1.5 N NaOH) dry basis

At all base concentration (0.5, 1.0, 1.5 and 2.0 N) of 10 and 20% of HCl acid and 0.5, 1.0 N of 30% HCl acid concentration, the product were brown and brownish white which indicated that pigments were present in the chitin and chitosan product. At 1.5 and 2.0 N of 30% HCl acid and all base concentration (0.5, 1.0, 1.5 and 2.0 N) of 40 and 50% HCl acid concentration, the products were white and good quality but this concentrations are economically expensive and the chemical waste are more threatening to the sound environment. But at 1.5 N base concentration of 30% HCl acid concentration, products (chitin and chitosan) are of good quality and white and these concentrations are economic and safe to environment as it leaves less residual acid to soil. Thirty percent is the best acid-base concentration for extraction of high quality final product.

The final products obtained from shrimp shells after 1.5 and 2 N of 30% and all samples of 40 and 50% acid treatment, the product are completely soluble in 1% acetic acid. It indicates that the products (chitosan) are of good quality. 0.5 and 1.0 N of 30% and all samples of 10 and 20% acid treatment, the products are slightly or half soluble in 1% acetic acid. The products are indicates that pigments are present in these products.

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