NOTE

ISOLATION AND CHARACTERIZATION OF CHITIN AND CHITOSAN PREPARED UNDER VARIOUS PROCESSING TIMES

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ABSTRACT

Generally production of chitosan from crustacean shells consists of 4 steps, i.e. deproteinization, demineralization, decolorization and deacetylation. Simplification of chitosan production by elimination of deproteinization and/or demineralization, or reducing of reaction time would give many advantages, e.g. reduction of processing time and cost production due to reduction of chemical and power usage. The objectives of this research were to prepare chitosan under various processing times and to characterize the obtained chitin and chitosan. Chitin was prepared under various deproteinization times (0, 15, 30 min at 90 ºC using NaOH 2N) and demineralization times (0, 15, 30 min at ambient temperature using HCl 2N). Chitin was then bleached using aceton/etanol (1:1) for an hour. Deacetylation was achieved by treatment of chitin under condition at 120 ºC for 5 hr using NaOH 50%. Ash and nitrogen content, and degree of deacetylation of chitosan were evaluated. Demineralization and/or deproteinization times influenced the quality of chitin. Chitin and chitosan prepared without demineralization had white and chalky appearance, whereas the other chitosan were off-white in color. Ash and nitrogen contents of the chitosan products were 0.18 – 32.40% and 3.56 – 7.59%, respectively. Chitosan prepared under various processing times, except chitosan without demineralization treatment, had degree of deacetylation ≥ 70%.

Keywords: *chitosan, deproteinization, demineralization, deacetylation, processing times*

INTRODUCTION

Chitin, the second most abundant biopolymer after cellulose, is the major component of crustacean shell such as prawn, shrimp, crab and crawfish. Chitosan is a natural biopolymer derived by deacetylation of chitin. During the past few decades, chitosan has been receiving increased attentions for its applications in medical, food and various chemical industries [1-2].This is based on the fact that chitosan is not only abundant naturally, but also nontoxic and biodegradable. Chitosan has been documented to posses an antimicrobial and antifungal activity [3-5] and a film-forming property for use as edible film and coatings [6-7]. Chitosan has also been documented to posses hypercholesterolemia functions [8].

Several studies have been conducted regarding the production of chitin and chitosan from various sources [3, 9-15]. The physicochemical characteristics of chitin and chitosan such as degree of deacetylation, molecular weight and solubility vary with crustacean species and preparation methods [16].

In the native material, chitin is associated with mineral, mainly calcium carbonate, protein, lipids including pigments. Typically production of chitin from crustacean shells consists of 3 basic steps, i.e. deproteinization, demineralization, and decolorization. Deproteinization is accomplished by extracting with sodium hydroxide solution at various temperatures (65-100 ºC) for 1-6 hr. Demineralization is achieved by extracting with hydrochloric acid solution at various temperatures (25-121 ºC) for 1 hour - 2 days. Pigment extraction or decolorization is accomplished by bleaching chitin in acetone/ethanol mixture or sodium hypochlorite solution. Conversion of chitin to chitosan is achieved by treatment with concentrated sodium hydroxide at various temperature, usually at 100 ºC or higher for a few hours. The production of chitin and/or chitosan is relatively expensive, thus limiting the application of chitosan. Simplification of chitosan production by eliminating deproteinization (DP) and/or demineralization (DM) step, or reduction of processing time would probably reduce the production cost because of the reduction of processing time, the reduction of chemical and power usage.

The aims of this research were to prepare chitin and chitosan under various processing times with the purpose of accelerating processing times, reducing the chemical and power usage and to characterize the obtained chitin and chitosan using several physicochemical methods.

EXPERIMENTAL SECTION

Material

The black tiger shrimp (*Penaeus monodon*) head shells were obtained from Semarang, Central Java, Indonesia. Wet shells were washed and dried under the sunlight for a few days. Dried shells were collected, packed in a big plastic bag and brought to the

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laboratory. Dried shells were crushed into pieces/flakes. This material was used throughout the research to obtain reproducibility and consistent results.

Procedure

Crushed shells were mixed with NaOH 2 N at a solid liquid ratio of 1:20 and deproteinised at 90 ºC for 0, 15 and 30 min with occasional stirring. This mixture was washed in running tap water, filtered to remove excess water, and left to dry at room temperature. Following deproteinization, the shell was demineralised with HCl 2 N at a solid liquid ratio of 1:20 for 0, 15 and 30 min at ambient temperature with occasional stirring. The residue was washed to neutral pH in running tap water, filtered and left to dry at room temperature. For every treatment, ash and nitrogen content were assayed and residue was weighed. The obtained chitin was then bleached using acetone/ethanol (1:1) for an hour. The decolorized chitin was washed using demineralised water, filtered, left to dry at room temperature, and packed in a plastic bag.

Deacetylation was achieved by treatment of chitin using NaOH 50% at 120 ºC for 5 hr at a solid liquid ratio of 1:20. The residue was washed, rinsed with demineralised water followed by ethanol 96%, filtered and dried at 50 ºC overnight. The obtained chitosan was collected, weighed and packed in a plastic bag. The chitosan was assayed for nitrogen and degree of deacetylation.

RESULT AND DISCUSSION

Chemical Composition of Shrimp Shell

Chemical analysis showed that moisture, ash and nitrogen content of the shrimp shells were 13.41%, 30.20%, and 4.53%, respectively (Table 1). Crustacean shells mainly consist of 30-50% of calcium carbonate, 30-40% of protein, and 20-30% of chitin on dry basis [17]. This composition varied depends on species and seasons.

Characteristic of Chitin and Chitosan

To compare whether there is any differences in ash and nitrogen content among various chitins prepared under various deproteinization and demineralization times, ash and nitrogen contents of chitin products were determined and the results are shown in Table 2. Ash content ranged from 0.99% to 30.20%. There was significant difference in ash content. The ash contents were different with various demineralization times. The longer demineralization times, the lower ash content of chitin products. However there is no significant difference between chitin with DP/DM 0/30 and 0/60 (data was not shown). Nitrogen content ranged from 2.72% to 4.53%. There was significant difference in nitrogen content. The longer deproteinization time, the lower nitrogen content of chitin product.

Table 1. Chemical composition of Black Tiger Shrimp (*P. monodon*) head shell

Composition	Content (%)
Water	13.41
Ash	30.20
Nitrogen	4.53

Table 2. Ash and nitrogen content of chitin

Treatment (DP/DM – min)	Chitin	
	Ash (%)	Nitrogen (%)
0/0	30.20c	-
0/15	2.34b	-
0/30	0.99a	-
0/0	-	4.53c
15/0	-	3.01b
30/0	-	2.72a

The appearance of the obtained chitin from every treatment was compared visually as shown on Fig 1. Chitin without demineralization step (DP/DM 15/0 and 30/0) had chalky appearance, whereas chitins with demineralization treatment were transparent. Chitins without deproteinization step (Fig 1d and 1g) were transparent and their colors were orange, whereas the color of the others was light pink. This is most probably due to the presence of protein and pigment on the chitin. Pigment removal was achieved by treatment using acetone/ethanol for an hour. The results clearly demonstrated that deproteinization and demineralization times influence the ash and nitrogen content of chitin products, and the final appearance of chitin products.

Ash and nitrogen content of various chitosan products were compared, and the result are given in Table 3. Ash and nitrogen content of the various chitosan products ranged between 0.13% and 32.40%, and from 3.56% to 7.59%, respectively. Marked differences in ash and nitrogen content were observed among the products. There was significant difference in ash and nitrogen content between chitosan with and without demineralization. Chitosan without demineralization had high ash content and low nitrogen content. In contrast, chitosan with demineralization had low ash content and high nitrogen content. Due to that condition, appearance of chitosan without demineralization was white and chalky, whereas the other chitosan was cream-white color.

Kim [10] reported that deacetylation is the process to convert chitin to chitosan by removal of acetyl group. There are several critical factors that affect deacetylation including treatments during chitin isolation, temperature and time of deacetylation, alkali concentration and its ratio to chitin. Degree of

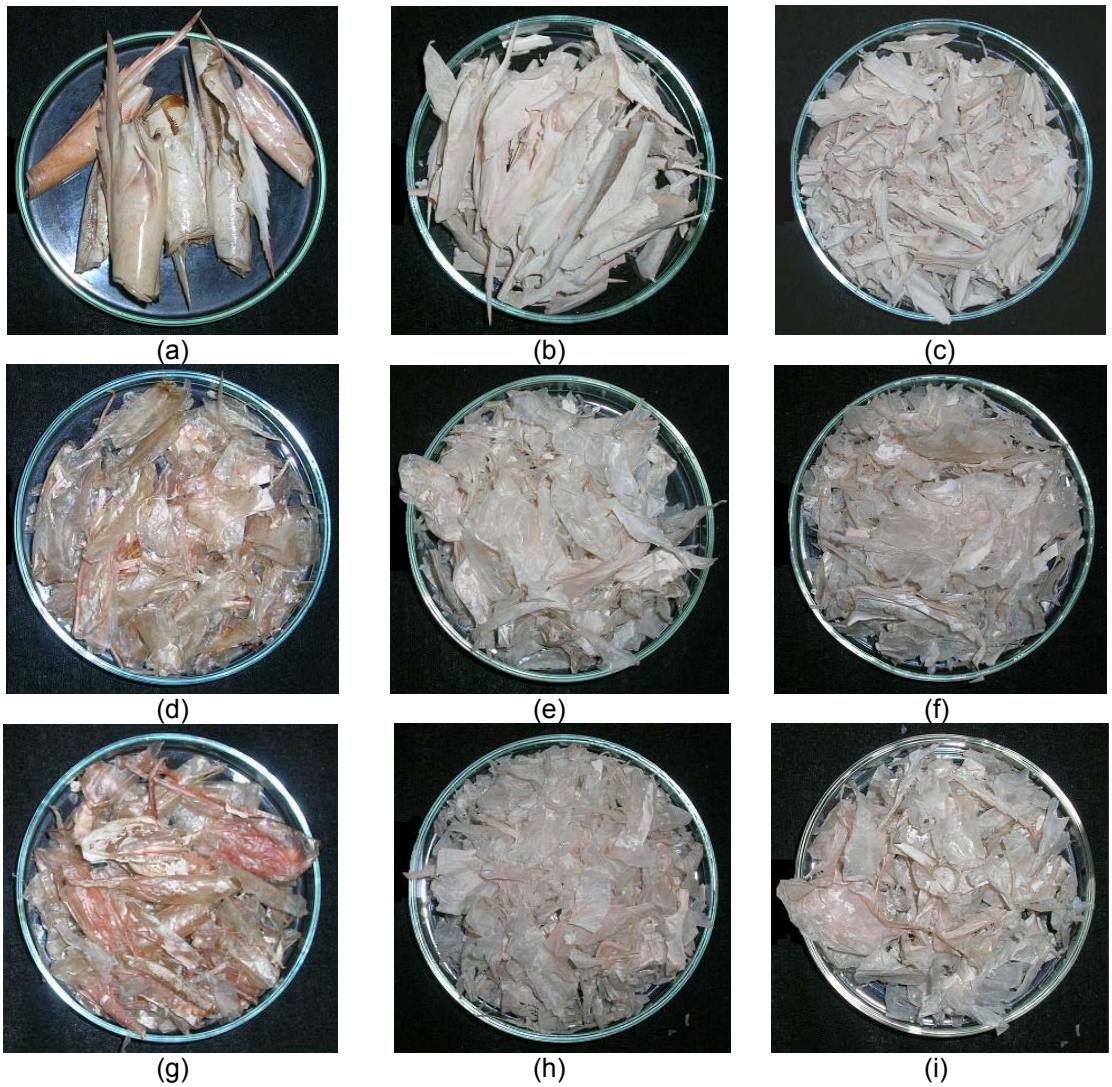

Fig 1. Appearance of chitin prepared under various deproteinization (DP) and demineralization (DM) times (a) DP/DM 0/0 (b) 15/0 (c) 30/0 (d) 0/15 (e) 15/15 (f) 30/15 (g) 0/30 (h) 15/30 (i) 30/30

Table 3. Ash, nitrogen and degree of deacetylation of chitosan

Treatment (DP/DM – min)	Chitosan		
	Ash (%)	Nitrogen (%)	Degree of deacetylation (%)
0/0	24.37^{c}	3.57^{a}	-
15/0	28.02^{d}	4.82^{b}	-
30/0	32.40^{e}	3.56^{a}	-
0/15	1.75^{b}	7.21^{cd}	76.14
15/15	0.26^{a}	7.39^{de}	79.13
30/15	0.19^{a}	7.15^{cd}	78.19
0/30	$0.8a^{b}$	7.59^{e}	79.28
15/30	0.13^{a}	7.19^{cd}	75.25
30/30	0.18^{a}	7.02^{c}	74.47

deacetylation is an important property of chitosan product because it affects the physicochemical properties of chitosan, hence it determines the application of chitosan. The degree of deacetylation of chitosan ranges from 70% to 96% depends on the crustacean species and the preparation methods [13].

Degree of deacetylation (DD) of various chitosan products was determined according to Khan [18] and

the results were shown in Table 3. Degree of deacetylation of chitosan without demineralization could not be determined. Due to high ash content presented in chitosan products, the acetyl groups probably still made a complex with minerals, such as calcium, hence could not be detected by the FTIR instrument. As a result the amide absorption band (1655 cm^{-1}) could not be shown.

Degree of deacetylation of chitosan with various demineralization and various deproteinization treatments was higher than 70% with the highest degree of deacetylation was 79.28%. There are some grades on chitosan product in commerce, i.e. industrial grade (DD ≥ 70%), food grade (DD ≥ 85%) and pharmaceutical grade (DD ≥ 90%). According to the results, the obtained chitosan could be classified as industrial grade chitosan.

CONCLUSION

This study was demonstrated that elimination of deproteinization and/or demineralization in chitin and chitosan preparation, or reduction of the processing time as an effort to reduce the production cost because of the reduction of processing time, chemical and power usage could influence the physicochemical properties of chitosan products. Chitosan prepared by simplification process can be used for specific application, especially for the application that does not require a pure chitosan, such as for coating of egg, fruit and vegetable. Further investigations are needed to study the physicochemical of the chitosan products such as solubility, viscosity, molecular weight and water/fat binding capacity in order to fit the application of chitosan products.

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