Research article

Effects of Chemical Extraction Methods on Physicochemical Properties of Shrimp Chitosan

SOCHEATH TONG*
Division of Research and Extension, Royal University of Agriculture, Phnom Penh, Cambodia
Email: tongsocheath@yahoo.com

BORARIN BUNTONG
Division of Research and Extension, Royal University of Agriculture, Phnom Penh, Cambodia

LAIKONG SOPHAL
Division of Research and Extension, Royal University of Agriculture, Phnom Penh, Cambodia

SOKMEAN VANN
Division of Research and Extension, Royal University of Agriculture, Phnom Penh, Cambodia

ANTONIO ACEDO JR.
Institute of Crop Science, University of the Philippines Los Banos, Laguna, Philippines

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Abstract Chitosan extraction methods have not been applied and optimized in Cambodia whose seafood industry produced tons of shrimp shell waste yearly. This study adopted the chemical extraction method and optimized the deacetylation step from chitin to chitosan using different concentrations of sodium hydroxide (NaOH). Shrimp waste (exoskeleton) was sequentially treated with NaOH (3.5%) for deproteination at 80oC; HCl (4%) for demineralization at 80oC; NaClO (0.315%) for decolorization at ambient temperature, and NaOH (40%, 50%, 60%) at 120oC for deacetylation. Chitosan yield, moisture content, total ash, lipid, fiber, solubility, nitrogen content, viscosity, and degree of deacetylation were determined. The commercial chitosan bought from a local market was included for comparison. NaOH at 50% was found to be the optimum concentration for deacetylation based on increased solubility (96.27%), reduced ash content (0.56%) and increased degree of deacetylation (83.23) as compared to that of 40% NaOH (93.61% solubility, 1.25% ash content, and 74.45 degree of deacetylation) though chitosan yield in the former (20.59%) was lower than that in the latter (25.23%). Increasing the NaOH level to 60% had no significant advantage. Lipid and fiber contents were not significantly affected. The characteristics of chitosan extracted with 50% NaOH at deacetylation stage were comparable to that of the commercial chitosan.

Keywords shrimp shell, chitin, chitosan, deacetylation, NaOH concentration

INTRODUCTION

Cambodia's seafood industry has long been faced with waste disposal problem, mainly shrimp shells from factories producing dried shrimps for local and export markets. Habitually, seafood waste is burned, land filled, dumped at sea or left to get spoiled. This has negative impact on the environment, biodiversity and human health. A sustainable solution is to transform shrimp shell waste into high-value products, particularly chitin and chitosan which have multiple applications in agriculture, food and beverage, medical, pharmaceutical. cosmetics and personal care, textile, environment, health, and water sectors. Global demand for chitin and chitosan is rapidly growing and is projected at 281,700 metric tons in 2027 (https://www.strategyr.com/market-report-chitin-and-chitosan-derivatives-forecasts-global-industry-analysts-inc.asp). However, global supply may remain short by over 50% of demand as in 2022 projections of global demand of 155,500 MT against supply of only 70,000 MT.

Chitin, a crystalline polysaccharide with high molecular weight, is the second most abundant organic compound after cellulose (Rinaudo, 2006; Kumari et al., 2015). It is considered as a natural fiber that is found in shells of marine animals, such as shrimps and lobsters, insect shells and in cell walls of several fungi and yeasts. In its structure, chitin is often linked to the other major constituents of the carapace, forming covalent bonds with 30-40% proteins and a complex matrix containing 30-50% calcium and phosphate carbonate and 20-30% of chitin. Extraction of chitin from shrimp shells involves at least two steps: deproteinization which removes proteins and undesired materials such as pigments and is often performed with dilute NaOH solutions, followed by demineralization which removes all minerals (e.g. $CaCO_3$) by using dilute HCl solutions. A decolorization step can be added to remove pigments and produce white product. The chitin produced is further processed by partial deacetylation to produce chitosan. Chitosan is a cationic semi-crystalline linear amino-polysaccharide of 1-4 linked N-acetyl glucosamine and glucosamine units; a white, hard, inelastic and nitrogenous polysaccharide (Cheba, 2011). It has three reactive functional groups, amine, primary and secondary hydroxyls that offer potential for covalent and ionic modifications allowing the improvement of its mechanical and biological properties. In addition, chitosan is a biodegradable, nonallergenic, biocompatible, antimicrobial, renewable, nontoxic product. Both chitosan and chitin have enormous economic value because of their flexible biological properties as well as their high nitrogen content (6.89%) as compared to synthetically substitute cellulose (1.25%). However, the crystallinity and insolubility of chitin demote its commercial applications.

The degree of deacetylation of chitosan ranges from 56% to 99% with an average of 80%, depending on the crustacean species and the preparation methods (No and Lee, 1995; No et al., 2000). Chitin with a degree of deacetylation greater than 50% is generally known as chitosan (Younes et al. 2014). In the deacetylation of chitin, several factors can influence the characteristics of resulting chitosan product. Rege and Block (1999) found that temperature and processing time were the most significant factors having noteworthy impact on degree of deacetylation and molecular weight. Tsaih and Chen (2003) concluded that molecular weight and deacetylation of chitosan are principally influenced by the concentration of NaOH, temperature, duration of reaction and recurrence of alkaline treatment steps.

OBJECTIVE

This study is a first attempt to harness available experience elsewhere on the chemical extraction of chitosan for shrimp shell waste of Cambodia's seafood industry and determined the effects of different NaOH concentrations during the deacetylation step producing chitosan from chitin.

METHODOLOGY

Chitosan Extraction

Figure 1 shows the process flow for chitosan extraction. Shrimp shell waste was collected from a local dried shrimp factory in Sihanoukville province, Cambodia, and brought to the Faculty of Agro-Industry, Royal University of Agriculture, Phnom Penh, Cambodia. The shells were properly washed with clean water prior to chitosan extraction which followed the procedures of Divya et al (2014) and Puvvada and Vankayalapati (2012).

The shrimp shells were deproteinized with 3.5% NaOH at 80oC for 3 hours with constant stirring at a solid to solvent ratio of 1:10 (w/v). Samples were then filtered under vacuum, and the filtrate was washed with tap water for 30 minutes and oven-dried.

Demineralization was performed by washing the deproteinized shells with 4% HCl for 4 hours at ambient temperature with a solid to solvent ratio of 1:10 (w/v), then filtered under vacuum. The filtrate was washed for 30 min with tap water and oven-dried.

The chitin produced was decolorized with 0.315% sodium hypochlorite for 15 min; with a solid to solvent ratio of 1:10 (w/v), followed by washing in tap water and drying under vacuum for 2-3 hours until the powder was crispy.

Deacetylation of chitin to produce chitosan was done using 40, 50 or 60% NaOH for 3 hours at 120oC with a solid to solvent ratio of 1:10 (w/v). The resulting chitosan was washed to neutrality in running tap water, rinsed with distilled water, filtered, and dried at 60oC for 24 hr in the oven.

Chitosan yield was calculated by comparing the weight measurements of the raw material to the chitosan obtained after treatment (Kiruba et al., 2013).

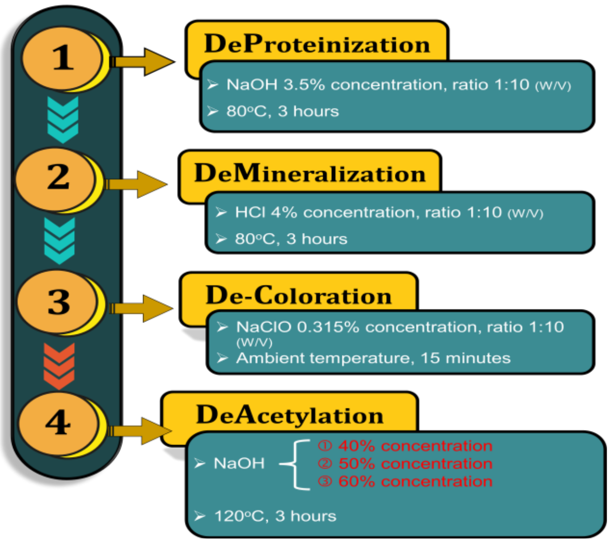

Fig. 1 Process flow for the extraction of Chitosan from shrimp shell waste

Proximate Analysis

Moisture, fiber, fat and nitrogen contents were analyzed following the AOAC (2001, 2005) methods. Total ash content was determined by the method of the Food Safety and Standards Authority of India (2012). Commercial chitosan (Sigma) was included in all relevant analysis as control.

Degree of Deacetylation

The titration method of Czechowska-biskup et al (2012) was followed. Dried chitosan (0.2 g) was dissolved in 20 ml 0.1 M hydrochloric acid and 25 ml deionized water. After 30 minutes continuous stirring, next portion of deionized water (25 ml) was added followed by stirring for 30 minutes. When chitosan was completely dissolved, solution was titrated with a 0.1N sodium hydroxide (NaOH) solution using automatic burette (0.01 ml accuracy). Degree of deacetylation (DA) of chitosan was calculated using formula (1):

$$DA[\%] = 2.03 \times \frac{V_2 - V_1}{m + 0.0042\ (V_2 - V_1)} \qquad (1)$$

where m: weight of sample, V_1, V_2: volumes of 0.1 N NaOH corresponding to the deflection points, 2.03: coefficient resulting from the molecular weight of chitin monomer unit, 0.0042: coefficient resulting from the difference between molecular weights of chitin and chitosan monomer units.

Solubility

Solubility of chitosan was determined by placing chitosan powder (0.1 g in triplicate) into a centrifuge tube (known weight) then dissolving in 10 ml of 1% acetic acid for 30 min using an incubator shaker operating at 240 rpm and 25°C. The solution was then immersed in a boiling water bath for 10 minutes, cooled to room temperature and centrifuged at 10,000 rpm for 10 min. The supernatant was decanted. The undissolved particles were washed in distilled water (25 ml) then again centrifuged a 10,000 rpm. The supernatant was removed and the undissolved pellets dried at 60°C for 24 hours. The dried pellets were weighed and the percentage solubility was calculated as follows:

$$\frac{[(\text{Initial weight of tube + chitosan}) - (\text{Final weight of tube + chitosan})] \times 100}{(\text{Initial weight of tube + chitosan}) - (\text{Initial weight of tube})} \quad (2)$$

Viscosity

Viscosity of chitosan was determined with a viscometer. Chitosan solution was prepared in 1% acetic acid at a 1% concentration on a dry basis. Measurement was made in duplicate using a No. 5 spindle at 50 rpm on solutions at 25°C with values reported in centipoises (cPs) unit.

Statistical Analysis

A completely randomized design with three replications was used. Data were subjected to analysis of variance and treatment mean comparison by least significant difference (LSD) test using the SPSS Statistical Package.

RESULTS AND DISCUSSION

In general, higher deacetylation degree increases the better solubility and viscosity of chitosan, the better chitosan quality. Deacetylation with 40% NaOH resulted in highest chitosan yield of about 25.2% (Table 1). Increasing the concentration of NaOH to 50-60% significantly decreased chitosan yield to about 20.6%. However, the degree of deacetylation was lower with 40% NaOH (74%) than with 50-60% NaOH (83%), the latter was comparable to that of commercial chitosan. Similarly, deacetylation with 50-60% NaOH significantly increased the solubility and viscosity of chitosan which were comparable to that of the commercial chitosan. Chitosan produced using 40% NaOH had the lowest solubility and viscosity. Chitosan produced with 50% NaOH had moisture, ash, fiber and nitrogen contents which were statistically comparable to that of the commercial chitosan (Table 2). With 40% NaOH, the chitosan produced had significantly higher ash and fiber contents and lower nitrogen content than the commercial control. NaOH at 60% had similar effects as 50% NaOH except that it had the lowest moisture content among treatments. Total fat content did not vary with treatment and ranged from 1.12-1.49%

Table 1 Physicochemical characteristics of chitosan extracted from shrimp shells using different NaOCl concentrations for deacetylation

NaOH concentration	Yield of chitosan (%)	Degree of deacetylation	Solubility (%)	Viscosity (log mPa/s)
Commercial chitosan	-	87.38a	95.65a	3.17b
40% NaOH	25.23a	74.45b	93.61b	2.84c
50% NaOH	20.59b	83.23a	96.27a	3.20a
60% NaOH	20.63b	83.35a	96.29a	3.18ab
Probability	**	**	**	**
LSD (5%)	0.882	5.342	0.287	0.022
CV (%)	2.00	3.00	2.00	0.00

Mean separation within columns by LSD, 5%. a, b, c Duncan's multiple range test

The results indicate that 50% NaOH was the optimum concentration for deacetylation of chitin to produce chitosan. The chitosan produced had degree of deacetylation, solubility and viscosity of 83.23%, 96.27% and 3.20 log mPa/s, respectively, and proximate composition of 11% moisture content, 0.56% total ash, 1.33% total fat, 1.47% total fiber and 7.29% nitrogen content which were statistically similar to that of commercial chitosan. Previous studies have successfully extracted chitosan from shrimp shell waste (e.g. Kandile et al., 2018; Nacer et al., 2019; Islam et al., 2020; Rasweefali et al., 2021) with similar results as the present study except for the lower chitosan yield obtained (Divya et al., 2014; Puvvada and Vankayalapati, 2012). Using lower NaOH concentration for deacetylation may increase yield of chitosan but other physicochemical properties were compromised. On the other hand, higher NaOH concentration gave no added benefit and may only increase the cost of extraction.

Table 2 Proximate analysis of chitosan extracted from shrimp shells using different NaOCl concentrations for deacetylation

NaOH concentration	Moisture content (%)	Total ash (%)	Total fat (%)	Fiber (%)	Nitrogen content (%)
Commercial Chitosan	10.53ab	0.59b	1.12	1.33b	7.12%a
40% NaOH	11.78a	1.25a	1.16	1.78a	6.12b
50% NaOH	11.00b	0.56b	1.33	1.47ab	7.29a
60% NaOH	10.14c	0.55b	1.49	1.36b	7.34a
Probability	**	**	ns	**	**
LSD (5%)	0.208	0.132	0.365	1.563	0.287
CV (%)	9.00	10.00	13.00	1.00	0.00

Mean separation within columns by LSD, 5%. a, b, c Duncan's multiple range test

CONCLUSION

Deacetylation is the final and critical stage to produce chitosan from chitin, both of which were successfully extracted in this research. NaOH at 50% was optimum for the deacetylation step. The physicochemical properties of chitosan extracted with 50% NaOH were comparable to that of the commercial chitosan. Future research could delve into manipulation of other deacetylation conditions (e.g. temperature and duration of deacetylation) that could increase the yield of chitosan without affecting the physicochemical characteristics.

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