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Effect of Chemical Treatment on the Characteristics of Shrimp Chitosan

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Abstract

The quality of chitosan produced from shrimp shell depends on the conditions of the chemical extraction process, including the concentration of chemicals used, the soaking time and the sequence of the treatments for deproteination, decalcification and deacetylation. The results show that 4% NaOH is a suitable concentration for removal of protein at room temperature (25°C). When the process started with deproteination, decalcification with 4% HCl for 2 h is sufficient to get chitin with a low ash content. For the production of chitosan with high viscosity, the decalcification should precede the deproteination. Chitosan with a high degree of deacetylation can be obtained by multiple deacetylation at 40°C.

Keywords: chitosan, deproteination, deacetylation, demineralization, chitin, viscosity

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Introduction

Shrimp is the first of the top ten foods exported in the year 2000 by Thailand with a 15% export share (National Food Institute, 2001). It is exported fresh, chilled, frozen, boiled and frozen, dried or salted in brine and as a value-added product. The major markets for frozen shrimps are the U.S.A., Japan and Singapore (Department of Business and Economics, 1999). Shrimp industries generate large amounts of shrimp biowaste during processing, approximately 45-55% of the weight of raw shrimp. The waste is sold to feed mills at a low price. However, this biowaste can be used to produce value-added products because it is rich in protein, carotenoids and chitin.

Currently nearly all chitin and chitosan produced commercially are chemically extracted from crab, shrimp and prawn exoskeleton waste (Roberts, 1997). Chitin can also be produced from shell waste by fermentation with microorganisms or with the aid of enzymes (Rao, *et al*. 2000). Enzymatic deacetylation of chitin to chitosan has been accomplished at the lab scale, but is not yet available for industrial scale (Win, *et al*. 2000; Win, *et al.* 2001). The chemical extraction of chitin is based on demineralization (or decalcification) by acid and deproteination by the action of alkali. Chitin is deacetylated into chitosan in concentrated alkaline (Roberts, 1997). These chemical treatments unfortunately are quite aggressive, leading to damage to the final product, corrosion of the equipment and the generation of a large volume of environmental unfriendly waste. This study has been focussed on mild conditions for chemical treatment.

Chitin is a cellulose-like biopolymer consisting of unbranched chains of predominantly β-(1→4)-2-acetamido-2-deoxy-D-glucose (also named *N*-acetyl-D-glucosamine) residues. Its structure is shown in **Figure 1a**. It does not dissolve in standard polar and non-polar solvents. It is present in fungi, yeast, marine invertebrates and arthropods, where it is a principal component in the exoskeleton (Jeuniaux, 1996).

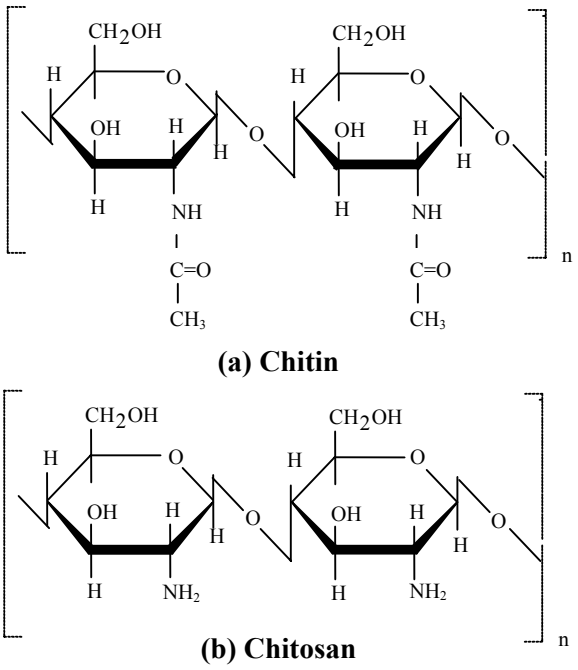


Figure 1 Structure of chitin and chitosan

Chitosan, the deacetylated form of chitin, is a polysaccharide formed primarily of repeating units of β-(1→4)-2-amino-2-deoxy-D-glucose (or D-glucosamine) and its structure is presented in **Figure 1b**. Chitosan is insoluble in most organic solvents and in water at neutral pH. However, it dissolves in acidic solutions (Hayes, *et al*. 1977). In this study the conditions for the extraction of chitin and chitosan from shrimp biowaste have been investigated with the aim to isolate chitosan with different physico-chemical characteristics including differences in viscosity and degree of deacetylation.

Experimental

Materials

Fresh local back tiger shrimp (*Penaeus monodon*) shells were used in this study and their composition was shown in **Table 1**.

Table 1 Composition of fresh shrimp shells

Parameter	value
Moisture content (%)	75.9
Ash content (%)	34.8
Protein (%)	27.7

Methods

Effect of serial treatment on characteristics of chitin and chitosan

Chitosan was prepared by two different treatments in which deproteination and decalcification were applied in either sequence. *Process 1*: Fresh shrimp shells without grinding were deproteinated with 1%, 2%, and 4% NaOH (ratio shrimp shell weight: volume NaOH = 1: 4.5 w/v)), at room temperature (25°C) for 21 h, then decalcified with 4% HCl (ratio = 1: 4.5 (w/v)), at room temperature for either 2 h or 12 h, followed by deacetylation with 50% NaOH (ratio = 1: 20 (w/v)), at 40°C for 3 days. The chitosan product was then washed with water until pH less than 7.5 and dried under sunlight. *Process 2*: Like the first process but the decalcification preceded the deproteination.

Determination of properties of chitin and chitosan

The yield of chitin and chitosan was determined based on oven dry weight of the initial sample. Degree of deacetylation (DD) was analyzed using HPLC (Ng, *et al.* 1996). HPLC analytical conditions ORH-801 cation exchange column elution with 1 mM H_2SO_4, 0.8 mL/min, column temperature 55°C. The ash content was calculated after heating the sample at 650°C for 3 h. The apparent viscosity of 1% chitosan was determined using a Brookfield viscometer "model DV-II+" (Lertsutthiwong, 1997). Protein content was analyzed by Biuret protein assay (Boyer, 1993) or by the microbiuret assay (Goa, 1953 cited by Bailey, 1967) depending on protein content in the sample. The solubility of chitosan was determined from residual weight of chitosan flakes after dissolving 1% chitosan in 1% acetic acid shaken at 200 rpm for 24 h.

Results and Discussion

Effect of serial treatment on the characteristics of chitin and chitosan

Chitin production: starting with deproteination

When the deproteination precedes the decalcification (Process 1), chitin becomes more white with increasing concentration of NaOH. Deproteination was carried out using 1%, 2% and 4% NaOH. The yield of chitin was 15-20% **(Figure 2**). The characteristics of the chitin are summarized in **Table 2**. The most significant effect of the variation of alkali concentration was the effect on the protein content. Decalcification with 4% HCl for only 2 h was enough to remove minerals (ash content was less than 1%). Prolonged decalcification time, even during 24 h, results in a very slight drop in the ash content but can cause polymer degradation (Brzeski, 1982 cited by No and Meyers, 1997).

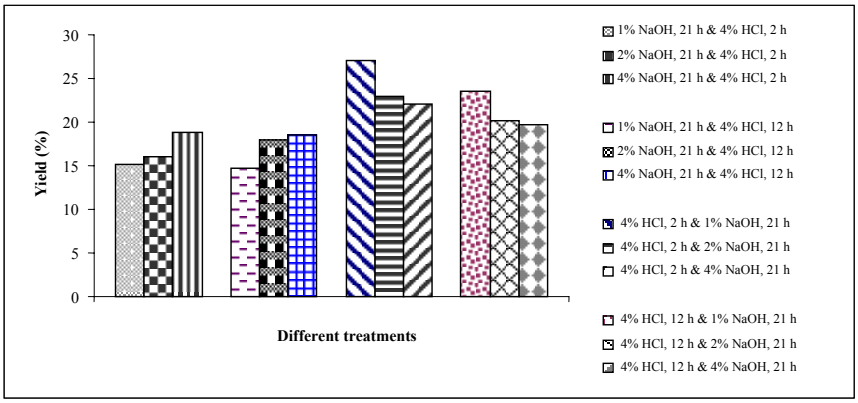


Figure 2 Yield of chitin produced by different serial treatments

The linkage between chitin part and protein moieties is probably through amide formation (Hackman, 1960 cited by Roberts, 1992). This formation occurs between the free amine groups in chitin with side chain carboxylate groups in the protein. In the presence of NaOH the amide is hydrolyzed and the protein content in the chitin product is reduced. Deproteination at room temperature should be done at 4% NaOH for 21 h to get a low protein content.

Table 2 Characteristics of chitin produced by different serial treatments

Treatments	Parameter			
	Moisture content (%)	Ash content (%)	DD (%)	Protein content (%)
1% NaOH, 21 h & 4% HCl, 2 h	7.93 ± 0.12	0.67 ± 0.06	13.1 ± 0.21	9.64 ± 0.26
4% HCl, 2 h & 1% NaOH, 21 h	8.06 ± 0.23	2.18 ± 0.09	23.1 ± 0.14	14.0 ± 0.21
2% NaOH, 21 h & 4% HCl, 2 h	7.38 ± 0.04	0.36 ± 0.03	10.2 ± 0.07	3.19 ± 0.12
4% HCl, 2 h & 2% NaOH, 21 h	7.75 ± 0.19	1.82 ± 0.06	11.3 ± 0.14	2.45 ± 0.00
4% NaOH, 21 h & 4% HCl, 2 h	8.08 ± 0.08	0.30 ± 0.02	9.05 ± 0.07	0.80 ± 0.04
4% HCl, 2 h & 4% NaOH, 21 h	7.37 ± 0.10	1.71 ± 0.09	6.50 ± 0.07	ND
1% NaOH, 21 h & 4% HCl, 12 h	7.84 ± 0.06	0.47 ± 0.05	14.3 ± 0.21	7.21 ± 0.08
4% HCl, 12 h & 1% NaOH, 21 h	7.84 ± 0.09	1.56 ± 0.09	24.9 ± 0.14	11.1 ± 0.09
2% NaOH, 21 h & 4% HCl, 12 h	7.90 ± 0.06	0.36 ± 0.02	9.20 ± 0.14	3.34 ± 0.09
4% HCl, 12 h & 2% NaOH, 21 h	7.95 ± 0.05	1.16 ± 0.02	9.70 ± 0.14	1.95 ± 0.09
4% NaOH, 21 h & 4% HCl, 12 h	7.27 ± 0.17	0.20 ± 0.01	8.35 ± 0.07	1.72 ± 0.10
4% HCl, 12 h & 4% NaOH, 21 h	8.03 ± 0.10	1.05 ± 0.05	7.20 ± 1.41	ND

Remark: *ND is non-detectable*

Chitin production: starting with decalcification

When fresh shrimp shells were decalcified first (Process 2) with 4% HCl for either 2 or 12 h, colored matter and protein still remained bound to the solid matrix. These materials are removed during the subsequent deproteination. Higher concentration of NaOH (4%) gave more efficient removal of protein and colored matter. The yield of chitin for most conditions was 20-27% (**Figure 2**).

The characteristics of chitin produced by this way are presented in **Table 2**. Ash content was 1-2%, protein content 1-2.5% for the 2% and 4% NaOH experiments. An increase in treatment time during decalcification or an increasing in the concentration of NaOH during deproteination resulted in a reduction in the ash content. Apparently, incomplete removal of protein increases the amount of minerals (Ca^{++}) that remains bound to the chitin solid fraction. The removal of protein should be carried out using 4% NaOH for 21 h.

Table 2 shows the differences between process 1 and process 2: The process that was started by decalcification gave higher ash contents than the process that was started by deproteination. It might be concluded that after deproteination, HCl is more effective in the removal of inorganic material than in the process started by decalcification.

If decalcification is carried out first, the yield is higher (**Figure 2**). This may be explained as follows. If deproteination is done first, the protective layer of protein is removed and the chitin that is unprotected is exposed to the HCl, leading to efficient demineralization but also to more hydrolysis and loss of material in the solid chitin fraction. If demineralization is done first, the chitin is protected by adhering protein, resulting in less hydrolysis of the backbone and in a higher yield. In the case of in the subsequent deproteination, 1% NaOH is used, the higher yield is partially explained by the large protein fraction in the chitin (14%, this is about one-seventh of the yield).

These data also suggest the effect of the sequence of treatments on the degree of deacetylation (DD) of chitin. If deproteination is carried out first, the DD of chitin is 8-14% (**Table 2**). This DD is considered to be the DD of the natural chitin. If demineralization is carried out first the calculated DD of chitin can be 7-25%. However, these higher DD values are the consequence of the high amount of protein, that contributes to the mass of the sample that does not contain acetyl groups and behaves therefore in the HPLC method as 100% deacetylated chitin. Corrected for this influence, all DD values are between 5 and 10%.

Effect of conditions during chitin extraction on subsequent chemical deacetylation to chitosan:

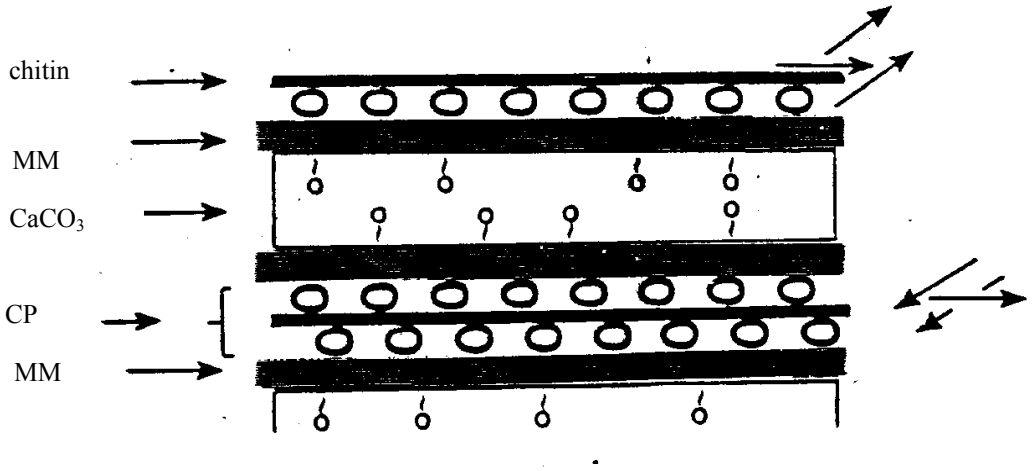
Chitin can be deacetylated to chitosan by treatment with 50% NaOH at 40°C for 3 days. The characteristics of the chitosan product are affected by the conditions of the chitin extraction. In case of deacetylation of chitin produced by deproteination prior to decalcification, the apparent viscosity of the chitosan depends on the concentration of NaOH during deproteination and on the duration of the decalcification with 4% HCl. A higher concentration of NaOH or longer duration of the treatment with 4% HCl gave a lower viscosity (**Table 3**). However, the quality of chitin did not affect the degree of deacetylation. The viscosity data can be explained by the sensitivity of chitosan for HCl. Longer HCl treatment leads to more hydrolysis and therefore to lower viscosity. In case of 1% NaOH, chitin contains 9.64% protein (**Table 2**). This protein protects chitin to hydrolysis, resulting in a higher viscosity of 830 cps. (compared to other viscosity values for chitosan obtained from chitin prepared with the deproteination first)

Table 3 Characteristics of chitosan derived from chitin produced by different serial treatments

Treatments	Parameter			
	Moisture content (%)	Ash content (%)	DD (%)	Viscosity (cps)
1% NaOH, 21 h & 4% HCl, 2 h	6.71 ± 0.10	0.51 ± 0.04	75.9 ± 0.35	830 ± 30
4% HCl, 2 h & 1% NaOH, 21 h	9.52 ± 0.16	0.89 ± 0.27	74.3 ± 0.07	5268 ± 146
2% NaOH, 21 h & 4% HCl, 2 h	6.96 ± 0.04	0.52 ± 0.04	75.7 ± 0.71	486 ± 14
4% HCl, 2 h & 2% NaOH, 21 h	8.13± 0.09	1.04 ± 0.08	75.5 ± 0.21	6370 ± 254
4% NaOH, 21 h & 4% HCl, 2 h	8.36 ± 0.06	0.50 ± 0.08	75.7 ± 0.28	435 ± 13
4% HCl, 2 h & 4% NaOH, 21 h	7.70 ± 0.14	1.01 ± 0.01	75.7 ± 0.85	5238 ± 190
1% NaOH, 21 h & 4% HCl, 12 h	7.44 ± 0.21	0.84 ± 0.08	73.2 ± 2.05	160 ± 9
4% HCl, 12 h & 1% NaOH, 21 h	12.6 ± 0.15	0.87 ± 0.04	74.8 ± 0.57	3420 ± 174
2% NaOH, 21 h & 4% HCl, 12 h	8.11 ± 0.15	0.71 ± 0.01	73.8 ± 3.0	110 ± 4
4% HCl, 12 h & 2% NaOH, 21 h	9.38 ± 0.04	0.96 ± 0.01	76.3 ± 0.07	2919 ± 93
4% NaOH, 21 h & 4% HCl, 12 h	7.97 ± 0.11	0.68 ± 0.02	74.1 ± 1.91	106 ± 5
4% HCl, 12 h & 4% NaOH, 21 h	8.33 ± 0.09	0.97 ± 0.01	76.1 ± 0.07	4470 ± 115

In case of the chitin produced by decalcification before deproteination, additional removal of ash forming material during the deacetylation procedure is observed, as shown in **Table 3**. Viscosity of this chitosan depends on the duration of the decalcification. Very high viscosity values were found. This demonstrates again the protective effect of the shrimp protein

layer against the hydrolytic action on the chitin backbone by HCl (Stevens, 2001). The schematic interpretation of this layer structure is shown in **Figure 3** (Poulicek, *et al.* 1986). It shows a thin layer of chitin sandwiched between two thicker layers of proteins. This complex is called a carrier protein (CP). The carrier protein is surrounded by the matrix of mineralized protein that is embedded in layers of $CaCO_3$. The protein in the mineralized layers and the protein in the carrier protein complex might protect the cleavage of the chitin chain against acid hydrolysis. In addition, a longer treatment time with 4% HCl resulted in a decrease of the apparent viscosity. The sequence of treatment did not show any effect on the degree of deacetylation.



$CaCO_3$ layers alternate with layers in which a chitin filament is covered with protein (CP) and is embedded in a mineralized proteinous matrix (MM). During deproteination, the protein is removed from CP; thereafter chitin is fully exposed to the chemical environment.

Figure 3 Schematic interpretation of the structure of the organic matrix in Mollusk shells (not drawn to scale) (Poulicek, *et al.* 1986).

Effect on Chitosan Quality

Chitosan: solubility, degree of deacetylation and ash content

Time and temperature of the chemical deacetylation reaction influence the properties of the chitosan product (Ng, *et al.* 2001; and Pajak, *et al.* 1998). The solubility of chitosan after 1 day of deacetylation with 50% NaOH (w/v) was higher than 90%. Solubility of the chitosan was measured after washing, drying and dissolution in 1% acetic acid. The amount of insoluble matter is probably related to chitosan particles with regions of insufficient deacetylation.

After 3 days of deacetylation at 40°C, over 70% of the acetyl groups in the chitin was removed (**Figure 4**). When the process of deacetylation was extended, the DD did not increase much any more. After 8 days of deacetylation, the DD obtained was about 80%. A higher degree of deacetylation, e.g. 85-90%, can not be achieved at low temperature in one step. Therefore the effect of double deacetylation at low temperature (40°C) on the degree of deacetylation was investigated. Chitosan with 88% DD could be reached when the deacetylation is carried out twice. The results are in agreement with those of Roberts (1997), and Chinadit, *et al.* (1998) who concluded that a multi-step process was required to obtain a high degree of deacetylation at low temperature. However, deacetylation at high temperature can reach high DD in one step as well. For instance, the deacetylation of chitin with 50% NaOH at 90°C for 5 h gave a chitosan product with 88% DD (Chinadit, *et al*. 1998).

Most of the $CaCO_3$ in shrimp shell is extracted during decalcification in 4% HCl. The residual amount of $CaCO_3$ is further removed in the process of deacetylation. **Figure 4** demonstrates that $CaCO_3$ in terms of ash content can be reduced to about 50% to a final value of 0.4%.

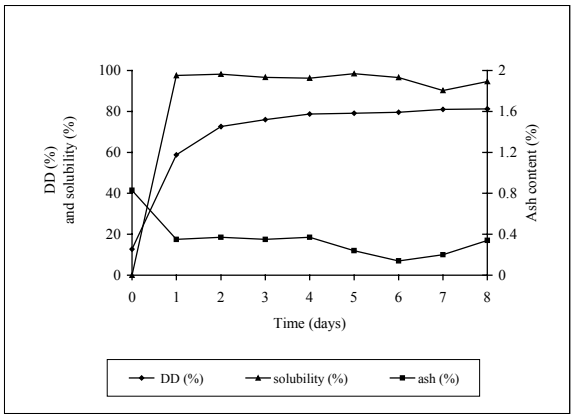


Figure 4 Effect of deacetylation time on the characteristics of chitosan.

Conclusion

Different chemical treatments in the production of chitin and chitosan result in variation in the characteristics of chitosan. To get chitosan with a high viscosity and a high degree of deacetylation at low temperature, the process should be started with decalcification and requires multi-deacetylation.

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