

# Improved Chitin and Chitosan Production from Black Tiger Shrimp Shells Using Salicylic Acid Pretreatment

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**Abstract:** Shrimp chitin and chitosan with improved characteristics were isolated from black tiger shrimp waste pretreated with 0.04 M Salicylic acid for ten hours. The pretreated shrimp shells could be efficiently demineralized and deproteinized at ambient temperature using 0.680 M HCl and 0.620 M NaOH, respectively. The duration of the treatments was 8 hours, the ash and protein residues in the final chitosan were about 0.48% and 0.51% respectively; the viscosity was 4800 cps; the solubility was up to 98%. In comparison with treatment at ambient temperature (30°C) without pretreatment, the chemical consumption, the duration of the treatment, ash and protein residues was reduced to 75-20%, whereas viscosity and absence of insolubles improved by a factor of 2.5.

**Keywords:** Chitin, chitosan, membrane, pretreated, Salicylic acid, wound healing management.

## INTRODUCTION

Chitin and chitosan are the structural components in the cuticles of crustacean, insects, mollusk and in the cell wall of fungi and plant pathogens (Suwalee, 2002) [1]. Reportedly, chitin and chitosan have various biological functions, for instance, antimicrobial activity; wound healing, especially on the proliferative phase and matrix formation.

Since the early eighties, chitin and its derivatives have been used for various biomedical purposes especially in skin and wound management products in Japan [2]. Though several standard chemical procedures have been widely used to produce chitin and chitosan utilizing shrimp shell waste as raw material, there is not much information about how to improve the production process so as to produce chitosan with consistent quality especially high solubility, high viscosity and high stability in degree of deacetylation while ash content ash and protein are as low as possible.

Salicylic acid is a keratolytic agent that softens the hyperkeratotic areas by dissolving the intra-cellular matrix and enhancing shedding of scales. This acid has been widely used in the removal of all types of human and animal skin warts using a technique of cauterization wherein slaked lime is applied to wart and then the surface of wart is scratched by using the stem of betel leaf, in general [3]. It has been shown that Salicylic acid softens keratin, loosens cornified epithelium and causes swelling even of viable cells. In a test of percutaneous absorption through skin of hairless rat as a good example, the data showed that Salicylic acid softens the horny layer and enhances the permeability of the stratum corneum.

Exoskeletal chitin-protein complexes give protection against chitinases and mild alkaline conditions. The more strongly the protein is bound to chitin, the harder for NaOH

to penetrate and to detach the protein from the solid shell material. Salicylic acid seems to weaken the exoskeletal complex matrix of protein resulting in the removal of a protein fraction that otherwise remains bound to the solid waste. As a consequence, this acid helps allowing NaOH solution to easily penetrate and facilitate the breakdown of protein components in the matrix.

This paper describes experiments to determine the suitable process for production of improved characteristics of chitin and chitosan from black tiger shrimp shells for various aspects of chitosan application.

## MATERIALS AND METHODS

The black tiger shrimp shells used in this study were obtained from a local shrimp processing factory, Southern part of Vietnam. The shells which were pretreated with 0.04M Salicylic acid was demineralized with 0.680 M HCl solution (1:5 w/v) at ambient temperature, 28-32°C for 8 hrs. The residue was washed and soaked in tap water for 6-8 hours. It was then dewatered and deproteinized with 0.620 M NaOH solution (1:5 w/v) at ambient temperature for 16hrs.

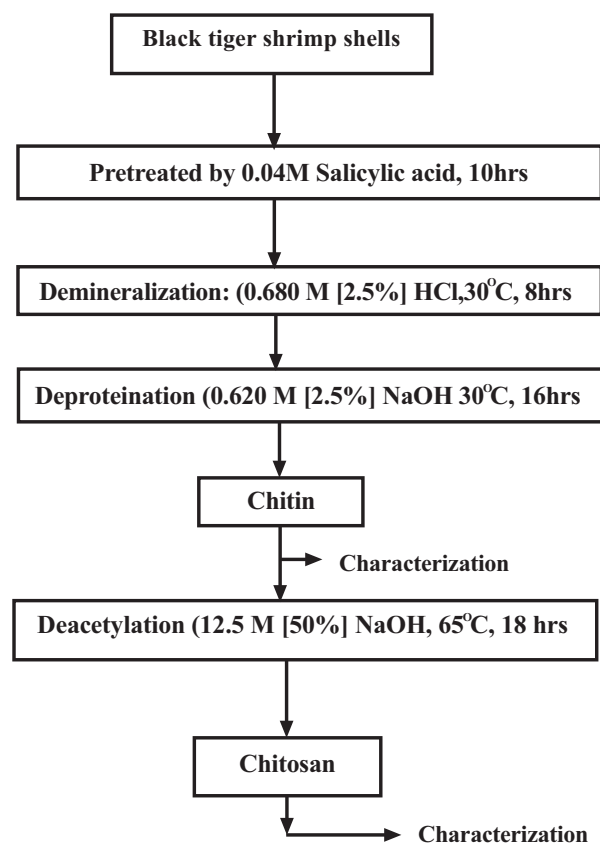
The chitin obtained from the above processes was deacetylated in 12.5 M NaOH (1:5 w/v) solution at 65°C for 18 hrs for chitosan isolation.

The process scheme for chitin/chitosan production under this study is shown in Fig. (1). After deacetylation, the chitosan was washed and dried in sunlight and assayed for moisture content, ash content, protein content, degree of deacetylation, viscosity, solubility, turbidity and molecular weight.

The protein content in the chitosan sample was determined using the micro-biuret method [4]. The degree of deacetylation was determined by first derivative ultraviolet (UV) spectrophotometry [5]. Weight average molecular weight was determined by gel permeation chromatography (Waters GPC) with a differential refractometer detector [6]. Dextrans of various molecular weights ranging from  $9.9 \times 10^3$  to  $2 \times 10^6$  were used as standards. Ash content was

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determined by the standard AOAC method [7]. Various physico-chemical criteria were investigated for chitosan in a 1% solution in 0.35 M acetic acid. Turbidity was assessed using a turbidimeter (Model 2100P, HACH Company, USA) and viscosity by a Brookfield Model DV - VII + Viscometer. Solubility was measured using the transglucosidase method [8]: the pH of 50 ml 1% (w/v) chitosan solution was adjusted to 4.8 with 30% (w/v) sodium acetate and mixed with Transglucosidase L-500 (Genencor International, 500  $\mu$ l). After incubating at 60°C for 24 hrs, the insoluble material was collected by filtration using a pre-weighed Whatman GF/C filter paper (1.2  $\mu$ m). The filter paper was dried and weighed and the amount of insolubles was calculated from its weight gain. Turbidity was assessed using a turbidimeter (Model 2100P, HACH Company, USA) and viscosity by a Brookfield Model DV-VII+ viscometer.



**Fig. (1).** The revised process for the production of chitin and chitosan under this study.

The reproducibility of chitosan was determined by storing chitosan in 0.1 M acetic acid for a period of 60 days at temperature of 28°C- 30°C according to method of Stevens [9]. In this study, the molecular weight was measured every ten days.

Chitosan membrane was tested for its thickness using a Mitutoyo micrometer. Tensile strength and elongation at break were measured for sample pieces of  $10 \pm 1 \mu$ m thickness (10 x15 mm) with a tensile tester, model Synergie 200, Adamel Lhmargy Co. Moisture sorption of chitosan membrane was measured at 84% relative humidity (Nunthanid *et al.*, 2001) [10]. Gel swelling index was determined by the method reported by Chen *et al.* (1994) [11]. Permeation of sodium salicylate through chitosan

membranes was assessed using a Franz diffusion cell (5 cm effective area of diffusion) at  $30 \pm 2^\circ\text{C}$ . The solute concentration in the receptor compartment was calculated from its absorbance at 296 nm using a spectrophotometer. Permeation coefficient was calculated by applying Fick's law. Lysozyme activity was assayed by the amount of reducing sugar generated by incubation of chitosan membrane (1 cm<sup>2</sup>) or chitosan powder (0.05 g) in a solution of 1.25 mg lysozyme in 25 ml 0.2 M phosphate buffer pH 7. The mixture was incubated at 37°C. The reducing sugar was determined after 24 h of incubation by the Schales method (Imoto and Yagishita, 1971) [12].

All data are expressed as means  $\pm$  standard deviation of representative of similar test carried out in triplicate. Statistical differences in cell number were determined by student's t-test in which,  $p < 0.005$  was considered statistical significant.

## RESULTS AND DISCUSSION

The general characteristics of chitin and chitosan produced from the pretreated shrimp shells by Salicylic acid are listed in Table 1.

**Table 1.** Characteristics of Chitin and Chitosan Produced from Pretreated Shrimp Shells by Dilute Salicylic Acid

Characteristics	Chitin	Chitosan
Ash content (%) <sup>*</sup>	$0.52 \pm 0.03$	$0.48 \pm 0.20$
Protein content (%) <sup>**</sup>	$0.55 \pm 0.04$	$0.51 \pm 0.25$
Solubility (%)		$98 \pm 0.03$
Degree of deacetylation (%)		$89 \pm 0.35$
Molecular Weight (daltons $\times 10^6$ )		1.71
Viscosity (cps)		$4850 \pm 300$
Turbidity (NTU) <sup>***</sup>		$22 \pm 5$

<sup>\*</sup>The ash content of the original shells was 24.5%

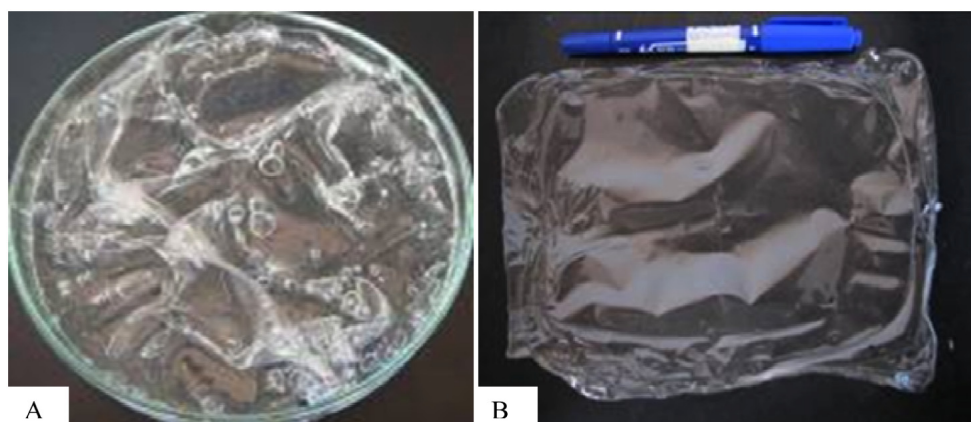
<sup>\*\*</sup>The protein content of the original shell was 24%

<sup>\*\*\*</sup>Chitosan 1% (w/v) dissolved in 0.35 mol L<sup>-1</sup> acetic acid (NTU: Nephelometric Turbidity Unit).

The viscosity of the Salicylic acid pretreated chitosan in solution is closed to 5000 cps compared with 2650 cps of control chitosan produced using common methods.

Data on the depolymerization of chitosan, of which, was storing in 0.1 M acetic acid for a period of 60 days at a temperature of 28°C- 30°C has been compared with studies of our colleagues in Bioprocess Program laboratory in Bangkok on comparison of storage of chitosan in acetic acid and in formic acid [9]. As expected, the produced chitosan using the proposed procedures under this study showed a reduction of less than 85% in Molecular weight (MW) in the 60<sup>th</sup> day. Data on the rate of chitosan degradation in 0.1 M acetic acid is shown in the Table 2.

The produced chitosan under the revised procedure was used for preparation of chitosan membrane and testing its characteristics, of which tensile strength, moisture sorption as well as the permeability constant are especially explored. As shown in Fig. (2) and Table 3, the chitosan membranes



**Fig. (2).** Appearance of chitosan membranes before and after detaching (A) and drying in the lab (B) at ambient temperature

were transparent and colorless. The average thickness of the membranes was  $10 \pm 1 \mu\text{m}$ .

**Table 2. Rate of Chitosan Degradation in 0.1 M Acetic Acid**

Time (Days)	1 <sup>st</sup>	10 <sup>th</sup>	20 <sup>th</sup>	30 <sup>th</sup>	40 <sup>th</sup>	50 <sup>th</sup>	60 <sup>th</sup>
Mw (Daltons $\times 10^6$ )	1.73	1.55	1.45	1.25	1.15	0.90	0.26

**Table 3. Characteristics of Chitosan Membrane from Chitosan of Deacetylation of 89%**

Chitosan Membrane	Characteristic Index
Crystallinity	$0.75 \pm 0.2$
Tensile strength	$2.35 \pm 0.4$
Moisture sorption	$70 \pm 7.0$
Permeability	$45.00 \pm 3$

**Notes:** Before performing the test, the chitosan membrane was preconditioned at  $25^\circ\text{C}$ , relative humidity of 50% for 24 hours.

Data of the current research are similar to those of Stevens [9] and Trung TS [6], provided that some characteristics of chitosan such as degree of deacetylation, solubility, turbidity and viscosity are essentially important parameters and should be taken into account before starting making chitosan membrane for further applications.

## CONCLUSIONS

The collected data demonstrate that the high and consistent quality of chitosan produced from black tiger shrimp shells could be obtained by pre-treating the shells using dilute Salicylic acid. Shrimp chitosan produced in this way could be used as a good source of raw material for further post processes of chitosan amongst other applications.

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