CHAPTER III
EXPERIMENTAL

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3.1 Materials and Chemicals

Shrimp shell (*Pandalus borealis*) was kindly provided by Surapon Foods Public Co., Ltd. (Thailand). Potassium hydroxide (KOH, analytical grade, Sigma Aldirch), glacial acetic acid (CH3COOH, analytical grade, J.T. Baker), Anhydrous sodium hydroxide pellets (NaOH, analytical grade, Univar), hydrochloric acid (HCl, analytical grade, ACI Lab Scan), N,N-dimethylacetamide (DMAc, ACI Lab Scan) methanol (CH_3OH, analytical grade, ACI Lab Scan) were used as received.

3.2 Methodology

3.2.1 Preparation of Chitin

Chitin was prepared from shrimp shell by decalcification and deproteinization to remove calcium carbonate and protein, respectively. First, the shrimp shells were cleaned and dried under sunlight for 3-5 days in order to remove the pigment out of the skeleton and then grinded into small pieces. Shrimp shell chips were treated by immersion in 1N HCl solution for 2 days with occasional stirring by changing the acid solution daily. After that, the decalcified product was washed with distilled water until neutral and dried in the 60°C until the product completely dried and weighed the decalcified product. Deproteinization was followed by boiling in 4 % w/v of NaOH solution at 80-90°C for 4 hr. The ratio of NaOH solution to shrimp shell was 10 to 1. After NaOH solution was decanted, the chips were washed with deionized water until neutral and dried in the 60 ° C until the product completely dried and followed by weighted. Decalcification step was repeat again by immersing in 1N HCl solution for 1 day, neutralized, dried and weighted ,to ensure that the calcium carbonate is completely remove the shrimp skeleton.

3.2.2 Preparation of Chitin Hydrogel

The preparation of chitin hydrogel are separated into three steps

3.2.2.1 Preparation of Calcium Chloride-saturated Methanol

Calcium chloride dehydrate 850 g was suspended in 1 l of methanol and refluxed solvent for 30 min to a near state of dissolution , followed by standing overnight at room temperature and subsequent filtration

3.2.2.2 Preparation of Chitin Solution

α-Chitin powder 20 g was suspended in 1 l Ca solvent and refluxed for several hours with stirring until chitin is completely disssolved , after that the insoluble material was removed by filtration.

3.2.2.3 Preparation of Chitin Hydrogel

Distilled water was added to 1 l of chitin solution with vigorous stirring and filtrate to collect the chitin precipitate. Secondly, centrifuge to separate water and chitin hydrogel ,followed by dialyzed chitin against distilled water to remove the calcium ions and methanol for 1 week and keep in the refrigerator before using.

3.3 Solution Plasma Set up

The solution plasma system was set up as shown in Fig. 1. The pulsed electric discharge was generated between two needle electrodes, made of tungsten, using a high frequency bipolar pulsed DC power supply. The two electrodes, of which the distance is 0.2 mm, are set inside a glass reactor where polymer solution is filled. Once the power is applied, the plasma was generated.

3.3.1 Deacetylation of Chitin Hydrogel by Solution Plasma

Chitin hydrogel (10 g of wet weight) was suspended in different concentrations of KOH in MeOH (0%, 7%, 10%). The suspension was then subjected to solution plasma to conduct the deacetylation reaction. The frequency, voltage and pulse width for solution plasma treatment were 15.0 kHz, 2.4 KV and 2 μs, respectively. The deacetylation reaction in KOH/MeOH via solution plasma was performed repeatedly up to 5 cycles and the plasma treatment time for each

cycle was 1 h. The deacetylation of chitin by solution plasma was done in comparison with the conventional heat treatment.

3.3.2 Depolymerization of Chitin Hydrogel by using Solution Plasma

Acetic acid solution (1% v/v) was used to depolymerize the chitin hydrogel by using solution plasma process. Thefrequency, voltage and pulse width for solution plasma treatment were 15.0 kHz, 2.24 kV and 2μs, respectively. After that the depolymerized products was precipitated in MeOH and washed until neutralbefore being subjected to deacetylation. The reaction times for depolymerization were varied to be 0h, 3 h and 5 h.

3.3.3 Chitosan Purification

The obtained products from deactylation and depolymerization were purified through dissolution in 1% acetic acid and centrifuged at 11000 in a centrifuge for 30 min in order to remove of insoluble material. For the precipitation of chitosan, occurred by addition of sodium hydroxide until pH 12.5, followed by neutralization until pH 7.0. The resulting chitosan suspension was centrifuged for separation of the supernatant.

3.4 Characterizations

3.4.1 Fourier Transformed Infrared Spectroscopy (FTIR)

The chemical structures of chitin and chitosan were determined by using FTIR spectroscopy (Thermo Nicolet Nexus, 670) at the wavenumber ranging from 4000 to 400 cm^{-1}. The degree of deacetylation (%DD) was calculated by using Baxter's equation (Baxter, Dillon, Taylor, & Roberts, 1992) as follows:

$$DD\ (\%) = 100 - [\ (A_{1655}/ A_{3450}) \times 115\ ] \qquad (1)$$

where A_{1655} is the absorbance at 1655 cm^{-1} of the amide-I band as a measurement of the N-acetyl group content and A_{3450} is the absorbance at 3450 cm^{-1} of the hydroxyl band as an internal standard.

3.4.2 X-ray Diffraction (XRD)

The chitin hydrogel and plasma treated chitin hydrogel was indentify the crystalline structures by XRD (Bruker AXS.D8 advance(and operated with the use of Cu Kα as an X-ray source. The scanning angle (2θ) from 5° to 50°

3.4.3 Gel Permeation Chromatography (GPC)

GPC was used to determine the weight-average molecular weight (Mw), number-average molecular weight (Mn) and molecular weight dispersion (Mw/Mn) of obtained product from deacetylation and depolymerization reaction. The obtained products were purified before using. The chitosan products were dissolve in acetate buffer at pH 5.5, a mixture of 0.1 CH_3COOH and 0.2 $CH_3CHOONa$, and filtrated through nylon 66 membrane with the pore size of 0.45 μm (Millipore, USA) before injection into the GPC instrument (Waters, Water 600E) equipped with an refractive index (RI) detector using an ultrahydrogel linear column Flow rate of mobile fate was set constant at 0.6mL min-1.The volumn of injection sample was 20 μL and chitosan concentration of 2.5 mg/mL at at 30 °C.