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Two-stage microfluidization combined with ultrafiltration treatment for chitosan mass production and molecular weight manipulation

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ABSTRACT

The objectives of the study were to propose a two-stage microfluidization combined with an ultrafiltration (UF) treatment for chitosan mass production and the manipulation of molecular weight and its distribution. The proposed methods are based on the degradation rate and rate constant of various process variables studied. Results obtained were that the rate constants were faster during the earlier reaction period, were higher for those operating at a higher pressure, were better for using concurrent UF treatment to remove small degraded fragments, and the degradation rate constants were faster for 30 °C solutions than that for 50 or 0 °C. A two-stage microfluidization process is proposed. The first stage constitutes of the highest possible concentration solution with concurrent UF treatment at 50 °C, and recycled 5 times. The second stage consists of the highest possible concentration of solutions with concurrent UF treatment at 30 °C, and recycled 5 times.

1. Introduction

Chitinous materials, including chitin and chitosan, are considered to be versatile, environmentally friendly biomaterials. Chitin is composed of *N*-acetyl-glucosamine and glucosamine. Chitinous materials, considered to be the most widely distributed polycationic biopolymers, have huge resources, are non-toxic and are biodegradable. Chitinous materials can be applied in food processing, agriculture, biomedicine, biochemistry, wastewater treatment, paper, textiles, cosmetics, nanoparticles, hydrogel, liquid crystals, membranes, microcapsules, etc. (Chang, Chang, & Tsai, 2007; Ravi Kumar, 2000; Rinaudo, 2006; Tsai, Bai, & Chen, 2008).

The molecular weight of chitinous materials is a very important parameter which affects their applications; therefore, it is important to develop a method by which to manipulate their molecular weight and also to preserve their integral structure. Degradation methods concurrently used include: chemical (Huang, Zhuo, & Guo, 2008), enzymatic (Li, Du, Liang, Yao, & Wei, 2006), physical methods of ultrasonic (Chen, Chang, & Shyur, 1997; Tsaih & Chen, 2003; Tsaih, Tseng, & Chen, 2004), microfluidization (Kasaai, Charlet, Paquin, & Arul, 2003), mechanical shearing (Chen, Chang, & Shyur, 1998), and microbial methods (Chen & Chen, 1999).

Chemical methods are difficult to manipulate with the molecular weights of resultant chitosan. Enzymatic and microbial methods cannot be feasible for use in mass production. The ultrasonic method is an efficient mechanical method; however, the eroded metal ions of the horn might contaminate the products. The mechanical shearing method apparatus is inexpensive and easy to obtain, but the degradation efficiency is low. Microfluidization is a potentially promising method which can be used in mass production and continuous processing.

The microfluidization process is one of the physical methods used to manipulate the molecular weight of the polymers. The solution stream was accelerated to a very high speed and forced into a reaction chamber by an air compressor. The process stream separates in two, changes direction and collides into a single stream again, generating a powerful shear force, turbulence, impaction, and cavitation forces. Those forces cause the disintegration of the particles or the degradation of the polymers (Cencia-Rohan & Silvestri, 1993; Kasaai et al., 2003; Silvestri, Gabrielson, & Wu, 1991). The microfluidization process is the combination of ultrasonic radiation and mechanical shearing. Microfluidization has been applied in cell rupture, homogenization or preparing the unilamellar vesicle (Masson, 1989).

Kasaai et al. (2003) reported that chitosan was degraded with microfluidization. The average number of chain scission, $(M_0/M_t) - 1$, was used as the index for degradation. The average number of chain scission increased bi-linearly with increased operation pressure, number of passes, and the molecular weight of chitosan used. However, the average number of chain scission decreased bi-linearly with the increase in solution concentration. The effect of solution temperature was not significant. The efficiency of chitosan degradation by continuous process was higher than that of volume passes. Chitosan degraded by microfluidization process will narrow the molecular weight distribution of the resulting product.

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The degree of deacetylation (DD) of the resulting chitosan increased when 0.1 M acetic acid was used as a solvent, whereas the DD of the resulting chitosan did not change when 0.04 M hydrochloric acid was used as a solvent.

Tsaih et al. (2004) reported that the 84% DD chitosan was degraded with ultrasonic radiation with or without concurrent ultrafiltration (UF) treatment used to remove the small degraded fragments. The rate constant and degradation kinetics were higher for those using concurrent UF treatment to remove small degraded fragments during the ultrasonic operation than for those not using UF ones, i.e. UF treatment can improve the de-polymerization rate of chitosan.

The objectives of this study were to propose a two-stage microfluidization process combined with an UF treatment for chitosan mass production and molecular weight manipulation. The proposed methods were based on the degradation rate and rate constant of various process variables, such as: number of recycled treatments, solution temperature, operation pressure, solution concentration, and with, or without, concurrent removal of degraded fragments during microfluidization.

2. Experimental

2.1. Chitosan preparation

Chitin was prepared from shrimp (*Solenocera prominentis*) waste by modifying a method of Stanley, Watters, Chan, and Mercer (1975) and Chen, Lin, and Yang (1994). Ground shrimp waste was treated with 0.5 N NaOH at ambient temperatures to hydrolyze the surface flesh. The alkali-treated waste was washed until it was neutral, and then dried and disintegrated to obtain powder. The powder was passed though sieves of 40–60 meshes. The flake-free powder was soaked in 2 N HCl for 2 h to remove the minerals, until no CO_2 evolved. The de-mineralized powder was soaked in 2 N NaOH at 80 °C to hydrolyze the protein, and then washed with water until neutral. The alkali-treated powder was soaked in 1% $KMnO_4$ at room temperature for 1 h to oxidize the astaxanthin, and then soaked in 1% oxalic acid at 80 °C for 1 h to neutralize the $KMnO_4$. This was washed then dried to get a white chitin powder. Chitin powder was alkali-treated (50% NaOH) at 140 °C for 3 h to get about 80% DD chitosan. This was washed until neutral and dried at 50 °C to get the final product (Tsaih & Chen, 1999).

2.2. Degree of deacetylation measurement

Infrared spectrometry was used to determine the DD of the chitosans (Baxter, Dillon, Taylor, & Roberts, 1992). Chitosan powder was strained through a 200 mesh sieve and then mixed with KBr (1:100) and pressed into a pellet. The absorbance of amide I (1655 cm^{-1}) and hydroxyl band (3450 cm^{-1}) was measured using a Bio-Rad FTS-155 infrared spectrophotometer. The band of the hydroxyl group at 3450 cm^{-1} was used as an internal standard to correct for disc thickness and for differences in chitosan concentration in making the KBr disc. The percentage of the amine group's acetylation in a sample is given by $(A_{1655}/A_{3450}) \times 115$. Here, A_{1655}, A_{3450} are the absorbance at 1650 and 3450 cm^{-1}, respectively. The DD of the chitosan used in this study is 84%.

2.3. Molecular weight determination

A size exclusion high performance liquid chromatography (SE-HPLC) method of Tsaih and Chen (1999) was followed. A column (7.8 mm × 30 cm) packed with TSK gel G4000 PW_{XL} and G5000 PW_{XL} (Tosoh Co., Ltd, Japan) was used. The mobile phase consisted of 0.2 M acetic acid/0.1 M sodium acetate, and 0.008 M sodium azide. Sample concentration of 0.1% (w/v) was loaded and eluated with a flow rate of 0.6 ml/min by an LDC Analytical ConstaMetric 3500 pump. The elute peak was detected by an RI detector (Gilson Model M132, USA). The data was analyzed by the Chem-Lab software (Scientific Information Service Corporation, Taiwan). Chitosans with known molecular weight (determined by light scattering) were used as markers. The calibration curve of the elution volume and the molecular weight were established. The weight average molecular weights of the samples were calculated from the calibration curve with the Chem-Lab software. The molecular weight of the chitosan used in this study was 1790 kDa.

2.4. Microfluidization treatment without concurrent removal of small degraded fragments

The chitosan solution was prepared by dissolving 0.2%, 0.8%, 1.4%, and 2.0% (w/v) of chitosan in acetic acid buffer (0.2 M acetic acid/0.1 M sodium acetate, pH 4.3). The solution was passed through a filter (Toyo Roshi Kaisha Ltd., No. 1, 55 mm, Japan) to remove the insoluble materials. An aliquot of 300 ml filtrate in a stainless vessel was placed in a water bath (Firstek, B403, Taipei) at a pre-set temperature of 0 ± 1, 30 ± 1, 50 ± 1 °C and was treated with a microfluidizer (Microfluidizer, M-100Y cell Disruption, Microfluidics Corporation, USA) at a pressure of 82.7 or 117.2 MPa for 5, 10, 15, 20 and 25 passes. An aliquot of the sample was piped out to analyze the molecular weight by SE-HPLC immediately after each preset cyclic treatment.

2.5. Microfluidization treatment with concurrent removal of small degraded fragments

During microfluidization treatment, the solution was circulated through an UF spiral-wound cartridge with a cut-off size of 1000 Da (Amicon CH2PRS system, Beverly, Mass.) to remove the degraded molecules (Fig. 1). The retentates were returned to the reactor for continuous treatment. Fresh solution equal to the volume of the eluate was added at the pre-determined time to make up the reaction solution at constant volume. An aliquot of the sample was piped out to analyze the molecular weight by SE-HPLC immediately after each preset cyclic treatment.

2.6. Calculation of the rate constant

The degradation reaction by microfluidization treatment is a first-order reaction. Its rate constant (k) can be obtained from

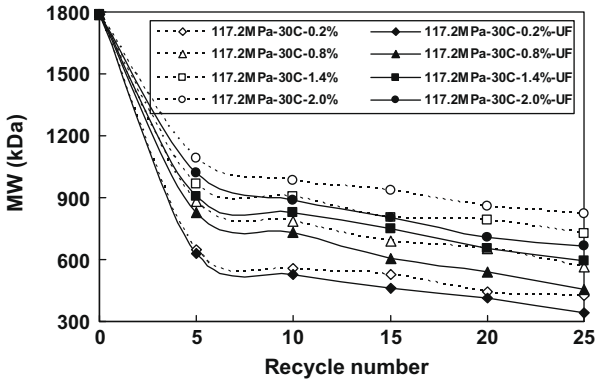

Fig. 1. Effect of solution concentration on the changes of molecular weight of chitosan treated by microfluidization with or without concurrent removal of small degraded fragments by ultrafiltration (UF) treatment over different cyclic treatments at 117.2 MPa, 30 °C.