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Depolymerization of chitosan–metal complexes via a solution plasma technique

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ABSTRACT

Chitosan–metal complexes were depolymerized under acidic conditions using a solution plasma system. Four different types of metal ions, including Ag^+, Zn^{2+}, Cu^{2+}, and Fe^{3+} ions, were added to the chitosan solution at a metal-to-chitosan molar ratio of 1:8. The depolymerization rate was affected by the types of metal ions that form complexes with chitosan. The complexation of chitosan with Cu^{2+} or Fe^{3+} ions strongly promoted the depolymerization rate of chitosan using a solution plasma treatment. However, chitosan–Ag^+ and chitosan–Zn^{2+} complexes exhibited no change in the depolymerization rate compared to chitosan. After plasma treatment of the chitosan–metal complexes, the depolymerized chitosan products were separated into water-insoluble and water-soluble fractions. The water-soluble fraction containing low-molecular-weight chitosan was obtained in a yield of less than 57% for the depolymerization of chitosan–Fe^{3+} complex with the plasma treatment time of 180 min.

1. Introduction

For chitin and chitosan, several studies have already been performed to prepare low-molecular-weight chitosan and their oligomers, which possess good biological activities, such as antitumor, antifungal, and antibacterial activities (Fernandes et al., 2008; Jeon, Park, & Kim, 2001; Kendra & Hadwiger, 1984; Liang et al., 2007; Muzzarelli, 2010; Qin et al., 2004; Toda et al., 1987) as well as the inhibition of metalloproteinase enzyme production, which can heal wounds and prevent wrinkle formation (Muzzarelli, 2009). In general, there are three widely used techniques for the depolymerization of chitin/chitosan, including chemical depolymerization, physical depolymerization, and enzymatic depolymerization. Among these three techniques, enzymatic methods have received more attention in recent years because they allow regioselective depolymerization of chitin/chitosan under the mildest conditions (Harish, Prashanth, & Tharanathan, 2007; Muzzarelli, Stanic, & Ramos, 1999). However, the important drawbacks of this technique are that the enzyme reaction progresses slowly and a low product yield is obtained (Choi et al., 2002). Most commercial enzymes, especially those with a high purity, are also expensive (Klaikherd et al., 2004). For chemical methods, various strong chemical reagents are used for the acid hydrolysis of chitin/chitosan leading to difficulty with handling. The generated wastes from these harmful chemicals may cause environmental pollution. Therefore, the development of new methods for the depolymerization of chitin/chitosan is of great interest.

Plasma in the liquid phase, which is known as "solution plasma", has recently been proposed to be one of the most effective strategies for the depolymerization of biopolymers, such as chitosan (Prasertsung et al., 2012) and sodium alginate (Watthanaphanit & Saito, 2013). However, most of the early research has been focused on the use of solution plasma, a glow discharge in the liquid phase, to synthesize metal nanoparticles with a narrow particle size distribution without adding reducing agents to the reaction system (Saito, Hieda, & Takai, 2009). Currently, the detailed structure of the solution plasma is still unclear but it is believed that the emission center of the plasma is located in the gas phase surrounded by a liquid phase, and an ion sheath is formed near the gas/liquid interface. This solution plasma capable of providing extremely rapid reactions due to the presence of activated chemical species and radicals under high pressure (Saito, Hieda, & Takai, 2009). Because the solution plasma is generated under mild conditions (i.e., the reaction proceeds at room temperature and without the use of strong chemical reagents) and is applicable to industrial material

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processing, this technique is very attractive for the depolymerization of biopolymers.

In the current study, the solution plasma was used for the depolymerization of an acidic chitosan solution. Various types of metal ions including the silver ion (Ag^+), zinc ion (Zn^{2+}), copper (II) ion (Cu^{2+}), and ferric ion (Fe^{3+}) were added to the chitosan solution to form chitosan–metal complexes because both hydroxyl groups (—OH) and amine ($—NH_2$) groups on the chitosan skeleton are good ligands for coordination with the metal ions. Coordination with the metal ions should result in weakening of the covalent bonds near the coordinating site resulting in weak points on the chitosan chain promoting the depolymerization reaction. A viscometric method and liquid chromatography were used to investigate the depolymerization process of chitosan. Any changes in the chemical structures and crystallinity of the depolymerized products were characterized by Fourier transform infrared (FTIR) spectroscopy and wide angle X-ray diffraction (WAXD) analysis, respectively. The molecular mass and degree of polymerization (DP) of the depolymerized chitosan were determined using mass spectrometry (MS).

2. Experimental

2.1. Materials

Chitosan was prepared from the shells of *Metapenaeus dobsoni* shrimp, which were provided by Surapon Foods Public Co., Ltd. (Thailand). Acetonitrile (CH_3CN), glacial acetic acid (CH_3COOH), hydrochloric acid (HCl), and sodium hydroxide (NaOH) pellets were purchased from RCI Labscan Limited (Thailand). A 50% (w/v) NaOH solution was supplied by the Chemical Enterprise Co., Ltd. (Thailand). Silver nitrate ($AgNO_3$) was purchased from Fisher Scientific (UK). Zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$) and copper (II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$) were provided by Ajax Finechem Pty Ltd. (Australia). Ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) and D-glucosamine hydrochloride (GlcN·HCl), which were used as a standard in the high performance liquid chromatography (HPLC), as well as high-purity distilled water, which was used as a solvent in the MS analysis, were supplied by Sigma–Aldrich (USA). Sodium borohydride ($NaBH_4$) was obtained from Carlo Erba Reagenti (Italy).

2.2. Preparation of chitosan from shrimp shells

M. dobsoni shrimp shells were cleaned and dried under sunlight prior to grinding them into small pieces. The ground shrimp shells were immersed in a 1 M HCl solution for 2 days with occasional stirring and were washed with distilled water until neutral. The demineralized shrimp shell chips were soaked in a 4% (w/v) NaOH solution at 80 °C for 4 h, followed by excessive washing with distilled water. The deproteinized product, or chitin, was subsequently deacetylated by heating in a 50% (w/v) NaOH solution containing 0.5 wt.% $NaBH_4$ in an autoclave at 110 °C for 75 min. After deacetylation, the chitosan flakes were washed with distilled water until neutral and were dried at 60 °C. The deacetylation step was performed twice to obtain chitosan with a high degree of deacetylation (%DD) (i.e., 90%), as determined by the FTIR technique reported by Baxter, Zivanovic, & Weiss (2005). A solid-to-liquid ratio used in the decalcification, the deproteinization, and the deacetylation steps was maintained at 1:10.

2.3. Solution plasma experiment

An aqueous solution of chitosan used in the solution plasma experiment was prepared by dissolving chitosan platelets in a 1% (w/v) CH_3COOH solution at a chitosan concentration of 0.5% (w/v). For the solution plasma experiment with metal complexation, $AgNO_3$, $Zn(NO_3)_2$, $Cu(SO_4)$, or $FeCl_3$ was dissolved in a 1% (w/v) acetic acid solution prior to dropwise addition to the chitosan solution with constant stirring at a metal-to-chitosan molar ratio of 1:8 and a final chitosan concentration of 0.5% (w/v). The solution mixture was maintained at room temperature (30 °C) for 5 h with constant stirring. Next, the chitosan solution either with or without complexation with metal ions was poured into the reaction vessel, which was a 100 mL beaker connected to two tungsten electrodes with a diameter of 1 mm. The solution plasma system used in this study has been described by Saito, Hieda, & Takai, 2009, Pootawang, Saito, & Takai (2011a), and Prasertsung et al. (2012). The operation parameters were maintained at an applied voltage of 1.44 kV, a pulse frequency of 15 Hz, a pulse width of 2.0 μs, and a gap distance of 1 mm. The reaction temperature during plasma treatment was approximately 80 °C. To determine the efficiency of the solution plasma technique for the depolymerization of chitosan, the acid hydrolysis of an aqueous chitosan solution in a 1% (w/v) CH_3COOH solution either with or without metal complexation at a reaction temperature of 80 °C served as a control.

2.4. Separation and purification of depolymerized products

An aqueous solution of chitosan sample after the depolymerization via both acid hydrolysis and solution plasma treatment either with or without metal complexation at different reaction times was collected prior to adjusting the solution pH to approximately 7 using a 5 M NaOH solution. The neutralized chitosan solution was maintained overnight at 4 °C for complete precipitation of the water-insoluble chitosan fraction (Choi et al., 2002). Then, the precipitate was removed by centrifugation at 8500 rpm at 4 °C for 20 min. The obtained supernatant was further mixed with an equal volume of acetone to yield a second precipitate, which is the water-soluble chitosan fraction (Choi et al., 2002; Prasertsung et al., 2012). The solution mixture was maintained overnight at 4 °C, and the second precipitate was collected by centrifugation at 8500 rpm at 4 °C for 20 min. After drying at 40 °C overnight, the water-insoluble and water-soluble chitosan fractions were weighed and used to calculate the percentage of water-insoluble and water-soluble chitosan fractions as well as the total yield percentage as follows:

$$\text{Water-insoluble chitosan}\ (\%) = \frac{W_i}{W_o} \times 100 \quad (1)$$

$$\text{Water-soluble chitosan}\ (\%) = \frac{W_s}{W_o} \times 100 \quad (2)$$

$$\text{Total yield}\ (\%) = \frac{W_i + W_s}{W_o} \times 100 \quad (3)$$

where W_i is the mass of the water-insoluble chitosan fraction after depolymerization, W_s is the mass of the water-soluble chitosan fraction after depolymerization, and W_o is the initial mass of chitosan in the acetic acid solution.

2.5. Analytical methods and measurements

As a preliminarily study of the depolymerization of chitosan, the viscosity of the chitosan solution was measured as a function of the reaction time with a Cannon-Ubbelohde viscometer (Cannon Instrument Co., J758). The viscometer was filled with a test sample and then equilibrated in a water bath at 25 °C. Then, the test solution was passed through the capillary once before measuring the running times at least in triplicate for each test sample. The running times of the solvent (i.e., a 1%, w/v, CH_3COOH solution) and the