Preparation, water solubility and antioxidant activity of branched-chain chitosan derivatives

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ABSTRACT

Water-solubility at neutral or basic pH of chitosan was largely improved by specific attachment of carbohydrates to the 2-amino functions achieved by Maillard reaction or further reductive alkylation of Schiff bases. The characteristic physicochemical, rheological properties, and antioxidant activities of the derivatives were investigated. Experimental results indicated that the solubility of all the chitosan-saccharides before and after reducing had been greatly enhanced comparing to the native chitosan. The Schiff base typed chitosan–fructose derivative was highest at 13.2 g/L of all, and Schiff base typed chitosan derivatives existed better solubility, Ph stability and more effective scavenging activity against DPPH radical than N-alkylated chitosan derivatives. The degree of substitution (DS) of the chitosan derivatives increased with higher concentration of saccharide, increasing reaction time and temperature. The reduction of viscosity of chitosan derivatives decreased with increasing reaction time and temperature. The results suggest that the water-soluble chitosan derivatives produced through Maillard reaction may be promising commercial additive in cosmetics and food.

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1. Introduction

Chitosan is a naturally occurring copolymer, one of the most abundant in the world, obtained from crustacean shells, insects, molluscan organs, and fungi (Jang, Kong, Jeong, Lee, & Nah, 2004). It consists unbranched chain of β-(1-4)-2-acetamido-2-acetamido-2-deoxy-β-ᴅ-glucose and β-(1-4)-2-amino-2-acetamido-2-deoxy-β-ᴅ-glucose as a repeating units. This polysaccharides has gained tremendous interest due to its excellent biological properties such as nontoxicity (Prabaharan, Borges, Godinho, & Mano, 2006), biodegradation (Sashiwa, Saimoto, Shigemasa, Ogawa, & Tokura, 1990; Shigemasa, Saito, Sashiwa, & Saimoto, 1994), biocompatible (Kurita, 1998), immunological (Song et al., 2009; Takashi et al., 1997), antibacterial (Tokura, Ueno, Miyazaki, & Nishi, 1997), and wound-healing activity (Kumar, Muzzarelli, Muzzareli, Sashiwa, & Domb, 2004). It has been widely applied in the fields of agriculture, environment, pharmaceuticals, medicines and industrial food processing (Chen et al., 2008; Ho, Mi, Sung, & Kuo, 2009; Liu, Nishi, Tokura, & Sakairi, 2001; Makoto et al., 2009; Zhang et al., 2009). However, chitosan-related applications are limited by its solubility only in diluted organic solutions such as formic, acetic, propionic, lactic, citric and succinic acid, as well as in a very few inorganic solvents, such as hydrochloric, phosphoric, and nitric acid at pH below 6.5 (Wang, Turhan, & Gunasekaran, 2004).

The intractability of chitosan lies largely in the rigid crystalline structure and the intermolecular hydrogen bonding caused by the acetamido or primary amino group residues (Nishimura, Kohgo, Kurita, & Kuzuhara, 1991). In an attempt to improve the water solubility of chitosan, many chemical modifications have been made to introduce hydrophilic groups by removing hydrogen atoms of the amino groups using acylation reaction (Sashiwa & Shigemasa, 1999), alkylation reaction (Chung, Tsai, & Li, 2006; Ma et al., 2008), quaternary reaction (Ignatova, Manolova, & Rasgkov, 2007; Verheul et al., 2008), carboxymethyl reaction (Sreedhar, Aparna, Sairam, & Hebalkar, 2007). Also there are other chemical and enzymatical modifications about chitosan (Sashiwa & Aiba, 2004).

The Maillard reaction is a well-known chemical reaction between an amino acid and a reducing sugar, usually requiring heat (Jokic, Wang, Liu, Frenkel, & Huang, 2004). Recently rheological characteristics and solubility of water-soluble chitosan derivatives derived from chitosan and saccharides have been demonstrated (Chung, Kuo, & Chen, 2005; Chung et al., 2006). The results indicate that the Maillard reaction is promising and facile for commercial manufacture of water-soluble chitosans. Thus, the development of a water-soluble chitosan is a prerequisite to successful industrial

application. However, the fundamental information reported is not enough. Considering the further application of chitosan to food and additives for skin care in the future, intensive research is worthy.

In this paper Schiff-base type derivatives and N-alkylation of chitosan with different degrees of substitution (DS) of various saccharides (aldoses or ketoses) was prepared by Maillard reaction and further reductive alkylation as facile and versatile procedure. For the sake of choosing the saccharide which could most improve the solubility of chitosan. A serious of mono-, di-, saccharides including D-glucose, D-fructose, L-rhamnose, D(+)galactose, L-arabinose, D-mannose, maltose, and D-lactose were systematically screened by a water-solubility-based screening method established to estimate the solubility of the chitosan derivatives qualitatively for the very first time. And D-fructose turned out to be the best. Solubility, antioxidation activity, reduction of viscosity and structure of chosen derivatives were characterized in detail.

2. Experimental

2.1. Materials

Chitosan: 90% degree of deacetylation (DD) MW 105 000 (Zhejiang Aoxing Biotechnology Co. Ltd., Yuhuan, China). The reagents used were of analytical grade.

2.2. Preparation of water-soluble chitosan

90% DD chitosan was dissolved in 0.2 mol/L CH_3COOH solution (pH 3.6) to give a final chitosan concentration of 0.056 mol/L. Reducing saccharides (aldoses or ketoses) was added to the solution to give a final concentration of 0.056 mol/L to 0.56 mol/L. 15 samples were reacted at 70 °C for 1–7 days. Every other day, 3 samples withdrawn were centrifuged (8000 rpm, 15 min) and dialyzed against distilled water for 4–6 days (pH 6–7). After qualitative analysis of the solubility, each sample was reduced by 10.0 eq. (–NH$_2$) sodium borohydride at room temperature for 24 h. The Schiff base samples with highest solubility and every reducing sample were dialyzed (membrane tubing, molecular weight cutoff 12 000–14 000, Spectrum Laboratories, Savannah, GA, USA) against distilled water for 4–6 days and then freeze-dried and weighed.

2.3. Determination of yield, solubility and solution stability

The yield of water-soluble chitosan (chitosan-saccharide derivative) was expressed as the ratio of water-soluble chitosan to total added chitosan and saccharides.

For the first time a water-solubility-based screening method was established to estimate solubility qualitatively: the absorbance of half the concentration of the reaction solution at 600 nm was assumed as A_1, the absorbance of the reaction solution after adjusting to pH 7.0 at the same concentration was assumed as A_2. The relatively smaller of the value of ΔA (=$A_2 - A_1$) was, the higher solubility of the sample would be.

To estimate solubility quantificationally, 0.05 g of water-soluble chitosan was mixed with 5 mL distilled water, stirred for 5 h and then filtered through a 0.45-μm filter paper. Solubility was estimated from the change in filter-paper weight (Yalpani & Hall, 1984).

To estimate the solution stability of the water-soluble chitosan, 0.1 g was dissolved in 10 mL distilled water. The pH of the solution was monitored by adding 2 mol/L NaOH solution drop-wise until the change of the absorbance of the solution at 600 nm was higher than 0.1, which was deemed unstable (Yang, Chou, & Li, 2002).

2.4. Determination of degree of deacetylation (DD) and degree of substitution (DS)

To determine DD or DS of the water-soluble chitosan, 20 mg of the soluble variant was dissolved in 10 mL acetic acid (0.1 mol/L) and completely stirred for 1 h at room temperature. The mixture was diluted with 40 mL distilled water, then 5 mL of the diluted solution was withdrawn and one drop of 1% toluidine blue added as an indicator. Potassium polyvinyl sulfate solution (PVSK, N/400) was successively added until the titration end point was reached (Toei & Kohara, 1976).

Since the consumption of the N/400 PVSK (A mL, Eq. (1)) might correspond to that a of glucosamine unit in the water-soluble chitosan, the total weight of a glucosamine unit (X g, Eq. (1)) in the solution was obtained by (Eq. (1)):

$$X = \frac{1}{400} \times \frac{1}{1000} \times F \times 161 \times A \tag{1}$$

where F is the factor of N/400 PVSK, 161 is the molecular weight of the glucosamine, and 203 is the molecular weight of the N-acetyl-D-glucosamine. The water-soluble chitosan unit (Y g) is expressed as (Eq. (2)):

$$Y = 0.5 \times \frac{1}{100} - X \tag{2}$$

Thus, DD is calculated by (Eq. (3)), DS is calculated by (Eq. (4)), 90 is the DD(%) of chitosan:

$$DD(\%) = \left[\frac{X/161}{X/161 + Y/203} \right] \times 100 \tag{3}$$

$$DS(\%) = \frac{90 - DD}{90} \times 100 \tag{4}$$

2.5. Determination of reactive extent of Maillard reaction

To assess the reactivity of the Maillard reaction, 3 mL solutions diluted 1 time from different chitosan-saccharide complexes were analyzed by measuring absorbance at 420 nm using a Beckman spectrophotometer (Liu, Chang, & Wu, 2003).

2.6. Evaluation of antioxidant activity

The DPPH (α,α-diphenyl-β-picryl-hydrazyl) scavenging activity of the samples was measured using the modified method of Yamaguchi et al. (Sun, Yao, Zhou, & Mao, 2008) 0.1 mL of ethanol solution of DPPH (0.1 mmol/L) was incubated with varying concentrations of test samples (0.1 mL). The reaction mixture was shaken well and incubated for 20 min at 30 °C and the absorbance of the resulting solution was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation:

$$\text{Scavenging effect } (\%) = \frac{1 - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

2.7. Determination of reduction of viscosity

The reduced viscosity of the N-alkylated chitosan derivative in 0.2 mol/L CH_3COOH/0.1 mol/L CH_3COONa was determined using an Ubbelohde-type viscometer (Schott-Gerate, Mainz, Germany) with a capacity of 15–20 mL. The viscometer was suspended in a thermostatically controlled water bath (Model E200, Lauda Dr. R. Wobser GmbH & Co., KG, Germany) maintained at 30.0 ± 0.1 °C. Flow times were recorded electronically using photoreceptors mounted on the viscometer stand which could detect the passage of the solution meniscus, and the solvent flow time ratio of the kinematic relative viscosity was thus obtained. Because of the low concentrations