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Effect of quaternization degree on physiochemical and biological activities of chitosan from squid pens

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ABSTRACT

Chitosan was prepared by alkaline *N*-deacetylation of β-chitin from squid pens, and *N*-(2-hydroxy) propyl-3-trimethyl ammonium chitosan chloride (HTCC) derivatives, with different degrees of quaternization (DQ) ranging from 0.77 to 1.06, were synthesized. It was identified by FT-IR, ^{1}H NMR and XRD analysis. All of the HTCC showed good water solubility in a wide pH range. The moisture absorption and retention abilities of all the HTCC were much better than that of the chitosan. The moisture absorption and retention values of all the HTCC at 43% RH for 24 h were above 49% and 92%, respectively. The scavenging ability of HTCC against hydroxyl and ABTS radicals improved with increasing concentration. The effectiveness of HTCC against hydroxyl radicals was lower than that of chitosan. These results indicated that HTCC, which has a much better moisture absorption and retention capacity, may act as a potential moisturizer in vitro.

1. Introduction

Chitin, the second most abundant natural polymer after cellulose, consists of partly deacetylated (1→4)-2-acetamido-2-deoxy-β-D-glucose units and is classified into α and β types [1]. Squid pens, the richest source of β-chitin, are currently regarded as the main waste component of squid [2,3]. Therefore, squid pens are valuable as a starting material for the preparation of β-chitin. Chitosan, a nontoxic copolymer consisting of β-(1,4)-2-amino-2-deoxy-D-glucose units, is obtained by *N*-deacetylation of chitin under alkaline condition [4,5]. β-Chitin exhibits higher reactivity and affinity toward solvents than natural α-chitin due to its much weaker intermolecular hydrogen bonding [6]. And chitosan prepared from β-chitin may have potential as a novel functional biopolymer and exhibit higher reactivity than that from α-chitin [7,8].

Chitosan has prospective applications in many fields such as biomedicine, wastewater treatment, functional membranes, food, tissue regeneration and cosmetic/personal care because of its desirable biological properties including biodegradation, biocompatibility, antimicrobial activity and antioxidant ability [9–12]. Unfortunately, the poor water solubility of chitosan has severely limited its processing and practical utilization.

To extend the utilization of chitosan, various chemical modifications such as carboxymethylation [7], lauroyl sulfonation [13], hydroxypropylation [14], and PEG-grafting [15], have been widely carried out. Compared with ordinary chitosan, chitosan derivatives have improved water-solubility over a wide pH range and obtained some special properties such as antioxidant activity, natural-derived emulsion stabilization, antitumor and antifungal activities [7,13–15]. Due to its enhanced water solubility and increased number of positive charges, quaternary ammonium chitosan has recently attracted considerable attention as bacteriostatic agent, textile finishing agent, drug delivery carrier [16,17]. Generally, there are two common ways to carry out quaternization of chitosan. *N*,*N*,*N*-trimethyl chitosan (TMC), the simplest form, is synthesized by reacting chitosan with methyl iodide under alkaline conditions and shows good antibacterial activity [16]. *N*-(2-hydroxyl)

Abbreviations: HTCC, *N*-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride; GTMAC, glycidyl trimethyl ammonium chloride; XRD, X-ray diffraction; DQ, degrees of quaternization; RH, relative humidity; ABTS, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid).

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propyl-3-trimethyl ammonium chitosan chloride (HTCC) is synthesized by the reaction of chitosan with glycidyl trimethyl ammonium chloride (GTMAC) [17]. The introduction of *N*-trimethylated quaternary ammonium salt group would greatly weaken the intermolecular hydrogen bonds, and thus will be helpful to improve the antibacterial activity, mucoadhesivity, moisture absorption and retention capabilities of chitosan [16–18].

Hyaluronic acid, an important functional moisturizer in cosmetics, is unique for its excellent moisture-retention ability, but the total amount is limited and the price is high [19]. Chitosans and their derivatives, the natural-derived hydrating agent, can act as a superior candidate in cosmetics because of their positive electrical charge and relative high molecular weight [20]. Reactive oxygen species (ROS) in the forms of hydroxyl radical, hydrogen peroxide and superoxide anion, have a wide variety of pathological effects, such as cancer, cardiovascular diseases, diabetes and atherosclerosis [21]. Antioxidants can reduce oxidative damage that is caused by ROS. Recently, the antioxidant activity of chitosan and its derivatives have attracted attention because of their nontoxic nature and natural abundance [7,14]. However, few studies on the antioxidant properties of HTCC prepared from squid pens are available. To the best of our knowledge, the degree of quaternary ammonium salt groups in the polymer chain will directly affect the properties of HTCC, and very few attempts have been made to examine the quaternization degree on the physicochemical and biological activities of HTCC. In this paper, chitosan was prepared by alkaline *N*-deacetylation of β-chitin from squid pens, and three quaternized chitosan (HTCC) derivatives with different degrees of quaternization (DQ) were synthesized in order to investigate their structural variation, moisture absorption and retention capabilities. As well, *in vitro* antioxidant activities of the derivatives were evaluated by scavenging hydroxyl and ABTS radicals.

2. Materials and methods

2.1. Materials

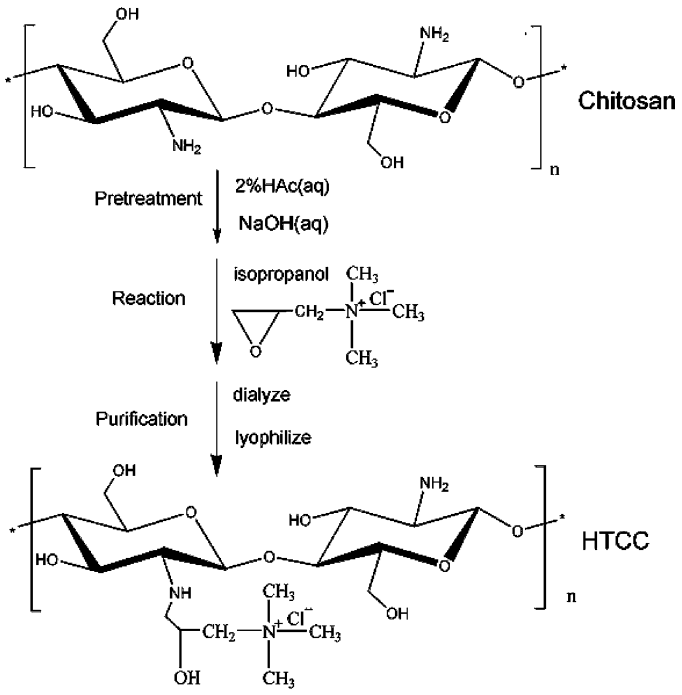
Squid pens were provided by Hangzhou Baokai Biochemical Co., Ltd. (Hangzhou, China). Sodium hydroxide, acetic acid, isopropyl alcohol, glycidyl trimethyl ammonium chloride (GTMAC), hydrogen peroxide (H_2O_2), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate ($K_2S_2O_8$), salicylic acid, bile acid (derived from taurocholate) and furfural were purchased from the Aladdin-reagent Co., Ltd. (Shanghai, China). All other chemicals were analytical grade and used without further purification. All water used in extraction and analysis was distilled and deionized.

2.2. Preparation of chitosan from squid pens

The preparation of chitosan from squid pens was carried out using the method of Huang et al. [22]. Chitosan with a degree of deacetylation of more than 90% was prepared from squid pens with a viscosity average-molecular weight of 6.5×10^5 Da.

2.3. Preparation of HTCC

HTCC was synthesized by a reaction of chitosan with GTMAC [23,24]. According to Scheme 1 and Table 1, 1.5 g chitosan was dissolved in 80 mL 2% acetic aqueous solution, subsided by dropwise adding 10% (w/w) NaOH solution and then kept at room temperature for 12 h. The precipitate was then washed with distilled water to neutral pH and transferred to a flask containing 60 mL isopropyl alcohol. After vigorously stirring for 1 h to evenly disperse the chitosan at 50 °C, the system was slowly heated to 60–80 °C, and by dropwise adding GTMAC/isopropanol solution (20% w/w) within



Scheme 1. Scheme of the preparation of water-soluble *N*-(2-hydroxyl) propyl-3-trimethyl ammonium chitosan chloride (HTCC).

1 h period. The molar ratio of GTMAC to $-NH_2$ in chitosan is 3:1. After that, the reaction was continuously stirred for another 6–10 h. The product was precipitated using isopropanol, dissolved in distilled water and then dialyzed using a regenerated cellulose tube (M_w cut-off 3500) against distilled water for 3 days. The resulting solution was subsequently concentrated by rotary evaporation at a reduced pressure at 50 °C and lyophilized to get purified HTCC product.

2.4. Characterization of HTCC derivatives

Fourier transform infrared (FT-IR) spectra of chitosan and HTCC were obtained using a Nicolet FT-IR spectrometer (Magna-IR 760 ESP, Nicolet Instrument Corp., Madison, WI, USA).

^{1}H NMR spectrum was recorded on a BRUKER DMX-500 Spectrometer. Samples were prepared by dissolving in D_2O/CF_3COOD mixing solvent.

The DQ of HTCC samples was defined as the proportion of hydrogen atom of $-NH_2$ being substituted by the quaternary ammonium salt group. And it was determined by titrating the amount of Cl^- ions with $AgNO_3$ aqueous solution, and was calculated as following:

$$DQ = \frac{VC}{VC + (m - VC \times 314)/162}$$

where C (mol/L) is the concentration of $AgNO_3$ solution, V (mL) the volume of $AgNO_3$ solution and m (g) is the weight of HTCC. The numbers 314 and 162 corresponded to the molecular weight of the repeat structural unit of HTCC and chitosan. There were two hydrogen atoms on each amino group which could be substituted by the quaternary ammonium salt groups [18]. When the hydrogen atoms of $-NH_2$ on chitosan were fully substituted, the DQ of HTCC was 200%.

X-ray diffraction spectra of the samples in the powder form were performed by a X-ray scattering diffractometer (X' Pert PRO) with Cu Kα 185 radiation (λ = 1.5444) in the range of 5–50° (2θ) at a voltage of 40 kV and a 186 current of 40 mA.