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Effects of hydroxypropyl degree on physiochemical activities of chitosan from *squid pens*

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ABSTRACT

Chitosan was prepared by alkaline *N*-deacetylation of β-chitin and hydroxypropyl derivatives with different degrees of substitution (DS) were synthesized. It was characterized by Fourier transform infrared (FT-IR) and elemental analysis. The DS of hydroxypropyl chitosan (HPCS) calculated by an element analyzer were 0.42, 0.75, 1.20, 1.82 and 2.25. HPCS showed better foam capacity and stability than that of chitosan, and the effectiveness correlated well with the DS of HPCS. The highest bile acid-binding capacity of all five HPCS reached 56.02 mg/g, which was 4.0-fold higher than that of chitosan. The scavenging ability of HPCS against hydroxyl and ABTS radicals improved with increasing concentration. The correlation between the hydroxypropyl content (DS) of HPCS and scavenging ABTS radical ability was positive. The hydroxyl radicals scavenging activity of HPCS correlated well with its increasing concentration, and EC_{50} values were below 12.5 mg/mL. These results indicated that hydroxypropylation is a possible approach to obtain chitosan derivatives with desirable physiochemical properties.

1. Introduction

Squid is a renewable natural resource and constitutes an important and profitable fishery in terms of both volume and economic value. Waste from the squid fishery accounts for 75% of the total weight harvested [1]. Squid pens, currently regarded as the main waste component of squid, are the richest source of β-chitin in the world [2]. Therefore, squid pens are valuable as a starting material for the production of β-chitin. Chitin, mainly classified into two types (α and β), is the second most abundant natural polymer after cellulose and consists of partly deacetylated (1→4)-2-acetamido-2-deoxy-β-D-glucose units. β-Chitin is characterized by much weaker intermolecular hydrogen bonding, and has been confirmed to have higher reactivity and affinity toward solvents than natural α-chitin [3–5].

Chitosan, the most important derivative of chitin, is a cationic polysaccharide obtained by *N*-deacetylation of chitin under alkaline condition [6–8]. It is noteworthy that chitosan prepared from β-chitin also exhibits higher reactivity than that produced from α-chitin [9]. Because of its excellent biological properties including biodegradation, biocompatibility, antimicrobial activity, antioxidant ability and non-toxicity, chitosan has prospective applications in many fields such as biomedicine, wastewater treatment, functional membranes, food, tissue regeneration and cosmetic/personal care [10–12]. However, chitosan is water insoluble, which severely limits its applications.

To extend the utilization of chitosan, various chemical modifications have been carried out such as carboxymethylation [4], quaternization [13], 6-amino-6-deoxydization [14], lauroyl sulfation [15] and hydroxypropylation [16]. Apart from its improved solubility, several other properties including its antioxidant activity, hemocompatibility and antimicrobial activity can also be improved.

Hydroxypropylation, a simple, low-cost, non-toxic, and water-soluble modification, has been shown to be effective in modifying the structure and properties of several polysaccharides including chitosan, starch, and cellulose [17]. Hydroxypropyl methylcellulose can be used as a functional excipient in solid matrix systems to prolong the release of active pharmaceutical ingredients [18]. Hydroxypropyl starch shows better swelling capacity with an increasing degree of substitution [19]. Hydroxypropyl cyclodextrin exhibits larger inclusion ability than cyclodextrin, and its complex shows higher scavenging capacity than a cyclodextrin complex [20,21]. Because of the unique amino group existed in chitosan, its hydroxypropyl derivative should display some unique

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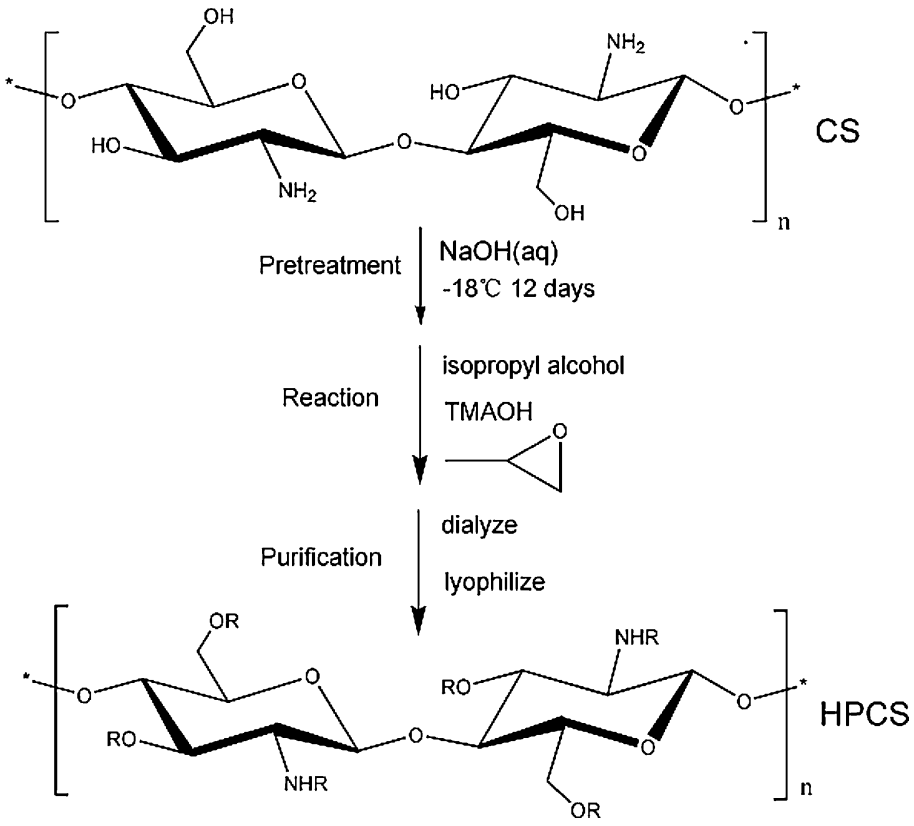

Scheme 1. Scheme of the preparation of water-soluble hydroxypropyl chitosan (HPCS), R=H or —$CH_2CH(OH)CH_3$.

properties compared with other polysaccharides. The recent studies focused on *in vitro* antimicrobial activity of HPCS with different degrees of substitution (DS) [16], the critical mesogenic behavior [22], the ionic conductivity and tensile properties of HPCS membranes [23], and the effect of HPCS on metal elements *in vivo* [24]. To date, the hydroxypropyl content of HPCS has not been evaluated for its potential in improving foaming properties, the antioxidant properties and bile acid binding capacities.

In this paper, chitosan was prepared by alkaline *N*-deacetylation of β-chitin from squid pens, and five hydroxypropyl chitosan (HPCS) derivatives with different DS were synthesized to investigate their foaming properties, *in vitro* bile acid-binding and antioxidant activities.

2. Materials and methods

2.1. Materials

Chitosan (CS) with a degree of 90% deacetylation was prepared from squid pens with a viscosity average-molecular weight of 6.52×10^5 Da. Sodium hydroxide, isopropyl alcohol, propylene epoxide, tetramethylammonium hydroxide (TMAOH), 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 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 HPCS

The preparation of HPCS was carried out based on the method that previously described [16,22]. According to Scheme 1 and Table 1, chitosan (2 g) was alkalized by adding 20 mL 15–45% (w/w) NaOH aqueous solution with stirring for 10 h at room temperature and kept at −18 °C for 0–12 days. The mixture was then thawed at room temperature and transferred to a flask containing 20 mL isopropyl alcohol. After vigorously stirring for 1 h at room temperature, 1 mL TMAOH was added and 35 mL propylene epoxide was added drop-wise with stirring over 1 h and the system was subjected to a continuous reaction at 40–64 °C for 4.5–9 h, using a reflux and condenser pipe. The resulting precipitate was neutralized by the addition of hydrochloric acid, and then dialyzed using a regenerated cellulose tube (M_w cut-off 3000) 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 obtain hydroxypropyl chitosan.

2.3. Characterization of HPCS derivatives

Fourier transform infrared (FT-IR) spectra of chitosan and HPCS were obtained with KBr pellets on a Nicolet FT-IR spectrometer (Magna-IR 760 ESP, Nicolet Instrument Corp., Madison, WI, USA).

CHN elemental analysis was performed on a Vario El cube CHNOS Elemental Analyzer (Elementar Co., Hanau, Germany). DS, which was designated as the average number of hydroxypropyl groups on each sugar residue, was calculated by C%/N% [22]:

$$DS = \frac{14}{36} \times \left[\frac{C}{N}(HPCS) - 5.14\right] \quad (1)$$

where 5.14 is the C/N value of chitosan as the deacetylation degree was 100%.

2.4. Bile acid-binding capacity assay

Based on the method of Muzzarelli et al. [25] with minor modifications, the bile acid-binding capacity of HPCS was measured