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Synthesis and preservative application of quaternized carboxymethyl chitosan containing guanidine groups

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ABSTRACT

A chitosan derivative, quaternized carboxymethyl chitosan containing guanidine groups (QGCMC), was synthesized and structurally characterized using FT-IR, ^{1}H NMR spectroscopy. The elemental composition of QGCMC was also investigated. Increase in the molar ratio of materials used for QGCMC synthesis and an increase in the reaction time of QGCMC synthesis led to an increased degree of substitution. QGCMC demonstrated a strong antimicrobial activity at acidic, neutral, and basic pH conditions and could significantly lengthen the shelf life of strawberries.

1. Introduction

The challenge of packaging food products with short shelf lives has received much attention during the past decades. Packaging of such food products using conventional materials based on petrochemical products is an effective means to lengthen their shelf life. However, serious environmental problems resulting from the excessive usage of petrochemical products has aroused social concerns [1]. In recent years, biopolymers, including starches, cellulose derivatives, chitin/chitosan, proteins, and lipids, have been considered as potential replacements for petrochemical products [2–5]. Biopolymer-based edible films and coatings present several advantages such as non-toxicity, negligible pollution, and low production costs. Their use could reduce water loss from the stored product and they might present other useful properties like anti-microbial activity or antioxidant activity, which would aid in extending the shelf life of the packaged product [6–9].

Chitin is the second-most abundant natural biological polysaccharide that is found in the exoskeleton of crustaceans such as crabs, shrimps, insects, and other arthropods [10]. The *N*-deacetylated derivative of chitin is chitosan, which can be dissolved in acidic solutions [11]. Chitosan is an important biopolymer because of properties like biocompatibility and biodegradability [12,13]. Furthermore, its degradation products are non-toxic, non-immunogenic, and non-carcinogenic [14]. The application of chitosan as a food preservative has been widely reported. Chitosan has been used in food-packaging films or coatings owing to its excellent film-forming ability. Furthermore, chitosan films are selectively permeable to gases (CO_2 and O_2) [21,22] and are thus able to reduce permeation of water vapor to some extent [23]. The antimicrobial activity of chitosan is attributed to its polycationic property [24]. Adherence to negatively charged residues at cell surfaces via the protonated amino group ($-NH_3^+$) is believed to be one of the mechanisms of antimicrobial activity of chitosan [25,26]. Furthermore, the number of protonated amino groups of chitosan, which increases with increasing degree of deacetylation (DD), is reported to influence its antimicrobial activity [27,28]. Poor solubility [29] and a limited pH range of antimicrobial action [30] have so far restricted the application of chitosan in the food industry.

A water-soluble derivative of chitosan, carboxymethyl chitosan (CMC), was synthesized to address the problem of its insolubility in neutral or basic solutions. CMC possesses excellent biocompatibility [15], high moisture retention ability [16], and is non-toxic. Owing to these properties, it has been widely used in biosensors [17] for wound healing [18], drug delivery [19], and food technology [20].

However, the antimicrobial activity of CMC is unsatisfactory [15]. To enhance the antimicrobial activity of CMC, *N*-halamine-modified chitosan was synthesized by Li et al. [31] and quaternized carboxymethyl chitosan was synthesized by Sun et al. and Wang et al. [32,33]. In spite of these modifications, the antimicrobial activities of chitosan and its derivatives are completely lost under neutral or basic conditions [25].

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Guanidine derivatives are another class of compounds that possess a broad-spectrum antimicrobial activity. They are highly alkaline compounds and exhibit low toxicity [34], owing to which they have found applications in medical [35], cosmetic [36], textile [37], and food industries [38]. Grafting of guanidine derivatives onto chitosan may enhance the antimicrobial activity of chitosan and extend the pH range of antimicrobial activity.

In this work, we introduced carboxymethyl, quaternary ammonium, and guanidine groups onto chitosan to improve its antimicrobial activity at a wider pH range and to increase its solubility in neutral or basic medium. The structure of the novel chitosan derivative, quaternized carboxymethyl chitosan containing guanidine groups (QGCMC), was characterized by FT-IR, ^{1}H NMR, and elemental analysis. Scanning electron microscopy (SEM) was used to examine the surface morphology of the film made from this derivative. Antimicrobial activity at different pH was also investigated. Finally, an effect of this derivative on preservation of food was evaluated.

2. Experimental procedures

2.1. Materials

Chitosan, possessing a deacetylation degree of 80–90% and viscosity of 50–800 mPa s, was purchased from Sinopharm Chemical Reagent Co., Ltd. Monochloroacetic acid, epoxy chloropropane, cyanamine (50% aqueous solution), and *N*,*N*-dimethyl-1,3-propanediamine were also purchased from Sinopharm Chemical Reagent Co., Ltd. All other chemicals were of reagent grade and used without further purification. *Escherichia coli* and *Staphylococcus aureus* were provided by Biological Experiment Center of Yancheng Institute of Technology.

2.2. Synthesis of QGCMC

2.2.1. Synthesis of CMC

CMC was synthesized according to the method of Sun et al. [32]. Briefly, chitosan (10 g) was mixed with NaOH solution (60 wt%, 15 mL), and stored overnight at −20 °C. Isopropanol (100 mL) was added to the frozen alkalized chitosan. Monochloroacetic acid ($ClCH_2COOH$, 30 g) was added into the isopropanol-containing chitosan solution. This solution was stirred at room temperature for 4 h, and the pH of this solution was adjusted between 7.0 and 8.0 using acetic acid. A solid precipitate of CMC was thus obtained, which was filtered and washed with acetone. Pure CMC was obtained after dialysis against ultrapure water for 3 days followed by vacuum-drying.

2.2.2. Synthesis of QGCMC

Cyanamine (3.9 mL) and *N*,*N*-dimethyl-1,3-propanediamine (6.5 mL) were dissolved in 50 mL of isopropanol. This mixture was stirred for 4 h at or below 10 °C, and the pH of this solution was controlled between 3 and 4 using HCl. An intermediate product, (IMP A), was obtained by liquid separation followed by an isopropanol wash. Following this, CMC (2 g) was allowed to react with epoxy chloropropane in water at 80 °C for 8 h. Another intermediate product, (IMP B), was obtained by distillation under reduced pressure followed by vacuum-drying. IMP B was then dissolved in 50 mL water and IMP A was added into this mixture very slowly while simultaneously controlling the pH between 4 and 5 using HCl at 31 °C. This mixture was further stirred for 8 h. The final product was dialyzed against ultrapure water for 2 days followed by decompression pressure distillation and vacuum-drying. Details of the synthesis are presented in Scheme 1.

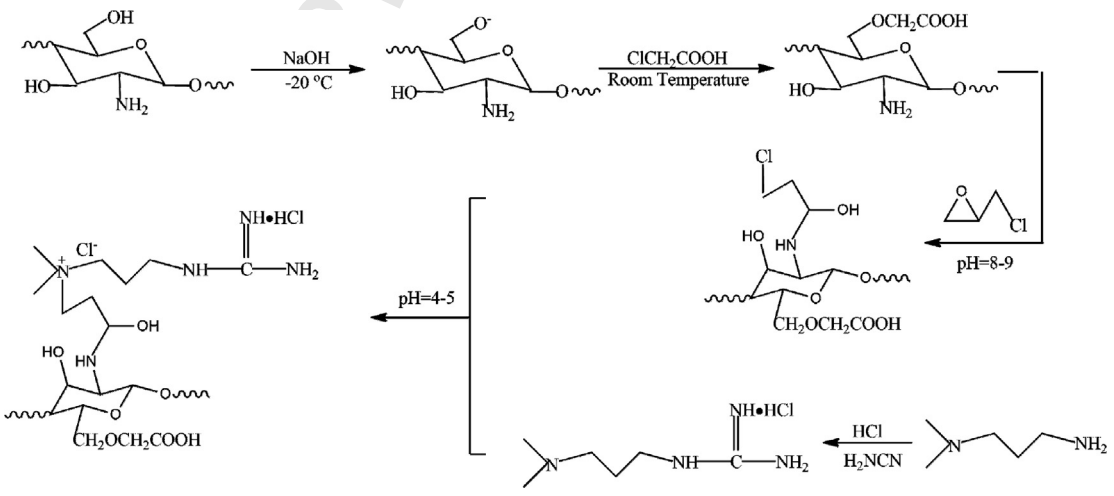
2.3. Structural characterization of QGCMC

FT-IR spectra were recorded with KBr pellets on a NEXUS-670 spectrophotometer (NICOLET, USA). ^{1}H NMR spectra were recorded on an AVANCE III 300M and chemical shifts are reported using methanol as a reference in D_2O at 293.4 K.

2.4. The effect of ratio of materials and reaction time on the degree of substitution

Quaternized CMC containing guanidine groups (QGCMCs) were synthesized using different molar ratios viz., 3:1, 2:1, 1:1, and 1:2, between IMP A and CMC. The molar quantity of epoxy chloropropane was similar to that of IMP A. The products thus obtained were marked as QG1-CMC, QG2-CMC, QG3-CMC, and QG4-CMC, respectively. The carbon, hydrogen, and nitrogen content of these products of CMC and of chitosan were determined using an elemental analyzer (Vario EL Cube, Elementar). The degree of deacetylation (DD), the degree of substitution of carboxymethyl (DSCM), and the degree of substitution of quaternized ammonium groups and guanidine groups were calculated according to Eqs. (1)–(3), respectively.

$$DD = 1 - \frac{1}{2}\left(\frac{14.01\omega_C}{12.01\omega_N} - 6\right) \quad (1)$$



Scheme 1. Synthesis of QGCMC.

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