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Physical properties and antibacterial activity of quaternized chitosan/carboxymethyl cellulose blend films

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ABSTRACT

Antibacterial property of packaging is needed to improve the microbiological safety of foods. The aim of this work was to incorporate various amounts of carboxymethyl cellulose (CMC) into films based on quaternized chitosan (2-*N*-Hydroxypropyl-3-trimethylammonium chloride chitosan, HTCC) to develop coating for preserving food. The HTCC film and blend films were characterized by Fourier transform infrared spectroscopy, X-ray diffraction measurements, scanning electron microscopy, and thermogravimetric analysis. The effects of CMC content on the physical properties and antibacterial activities of the blend films were investigated. The effect of coating bananas with the films on their preservation was also determined. The results revealed that HTCC and CMC in the blend films interacted by hydrogen bonding. Both of them could be partly miscible. Compared with HTCC100 film, CMC incorporation improved tensile strength, thermostability, and water resistance, however, increased oxygen permeability, and decreased light transmittance and antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria. Bananas coated with the HTCC/CMC blend films had longer shelf life than uncoated bananas. CMC incorporation thus evidently reinforced the HTCC film and lowered its WVP. These results suggest that HTCC/CMC blend films can be used as food packaging materials.
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1. Introduction

Food-packaging materials with proper mechanical properties, water and gas barrier properties, thermostability, antibacterial activity, and low environmental impact are highly desirable. Such materials retard deterioration, as well as maintain the quality and safety of foods (Brody, Bugusu, Han, Sand, & McHugh, 2008; Shankar, Teng, Li, & Rhim, 2015). Synthetic polymeric films are widely used in food packaging because they are inexpensive, convenient to produce from raw materials, and highly durable. However, these films can cause serious white pollution because they are non-biodegradable; therefore, there is increasing interest in the use of biodegradable alternatives from biopolymers. Biopolymers such as starch, agar, chitosan, carrageenan, and gelatin have been used for this purpose (Rhim, Park, & Ha, 2013).

Chitosan, an aminopolysaccharide that is abundant in nature, is composed of β(1 → 4) glucosidic bonds (Tsai, Hung, Lai, Wang, & Hsieh, 2014). Chitosan and its derivatives are biocompatible, biodegradable, and nontoxic, and they possess film-forming and antibacterial properties related to their polycationic structure (Xu, Wang, Guo, Lei, & Tang, 2011). However, chitosan is soluble only in acidic media and starts to lose its antibacterial activity at pH 6.5 (Mivehi, Hajir Bahrami, & Malek, 2008). In contrast, its quaternary ammonium derivatives have high activity against a variety of bacteria and are water soluble over a wide range of pH values (Huang, Li, Xue, Huang, Deng, & Ma, 2013; Tan, Peng, Li, Xu, Guo, & Tang, 2012). The antibacterial activity of quaternized chitosan is better than that of chitosan itself (Schreiber, Bozell, Hayes, & Zivanovic, 2013), but it exhibits strong hydrophilicity and poor mechanical properties, which makes it unsatisfactory for applications in food packaging. Its incorporation with other polymers may be an effective method for overcoming such drawbacks and for obtaining blend materials with desired functions and properties.

Carboxymethyl cellulose (CMC), a common derivative of cellulose, is a water-soluble, anionic polysaccharide (Dashipour et al., 2015). Its solution has good film-forming property and is nontoxic; thus, it is used as an additive to improve the product quality and processing properties of food packaging materials

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(Ma, Chang, & Yu, 2008). Studies on incorporation of CMC into matrices such as polyvinlypyrrolidone (Roy, Saha, Kitano, & Saha, 2012), polyvinyl alcohol (El Sayed, 2014), and konjac glucomannan (Cheng, Abd Karim, & Seow, 2008) have been performed to enhance the physical properties of chitosan films. Studies on the effects of addition of CMC on the properties of quaternized chitosan (2-*N*-Hydroxypropyl-3-trimethylammonium chloride chitosan, HTCC) films have not been reported.

The purpose of the present work was to evaluate the effect of incorporating CMC on the physical and antimicrobial properties of HTCC films. HTCC film and HTCC/CMC blend films were characterized by Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA). Tensile strength (σ_b-), oxygen permeability (OP), water vapor permeability (WVP), light transmission, as well as activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacteria were also measured. The appearance of bananas with and of those without film coating was observed to evaluate the preservation effect.

2. Material and methods

2.1. Materials

HTCC (>91% degree of substitution) with an average molecular weight of 50,000 was purchased from Tianhua Bio-Assistants Co., Ltd. (Shandong, China). CMC with 0.55–1.0° of substitution and 800–1200 mPa s viscosity (20 g L^{-1}, 25 °C) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All products were used without further purification. Other chemicals were of analytical grade. *E. coli* (ATCC25922-3) and *S. aureus* (ATCC25923-3) were obtained from Qingdao Hope Bio-Technology Co., Ltd. (Qingdao, China). Bananas with good uniformity were carefully selected from the same bunch. All other biochemical reagents were purchased from AoBoXing Bio-tech Co., Ltd. (Beijing, China).

2.2. Preparation of HTCC/CMC films

An HTCC solution (5% w/v) was prepared by dissolving HTCC in distilled water at room temperature with mechanical stirring at 500 rpm for 30min. A CMC solution (2% w/v) was prepared by dissolving CMC in distilled water at room temperature with mechanical stirring at 500 rpm until a homogeneous viscous solution was obtained. The film-forming solutions were obtained by mixing HTCC and CMC solutions by stirring (400 rpm) at room temperature for 30 min with different mass rations, degassed in a vacuum oven and then poured into a Teflon pane (30 cm × 30 cm × 3.5 cm). The solution in the Teflon pane was dried at 50 °C to obtain films with uniform thickness. The obtained films in different HTCC/CMC mass ratio were noted according to mass ratio of HTCC to CMC as follows: HTCC100, HTCC90/CMC10, HTCC70/CMC30, HTCC40/CMC60, CMC100.

2.3. Characterization

2.3.1. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded on a Nicolette 6700 spectrometer (Thermo Fisher Scientific Co., Ltd., MA, USA) with attenuated total reflection (ATR) mode at a resolution of 4 cm^{-1} in a range of wavenumber from 4000 to 700 cm^{-1}. These films were dried at 40 °C for 24 h and cut into 1 cm × 1 cm before the measurement.

2.3.2. X-ray diffractometery (XRD)

XRD patterns were obtained by using a D/max-2200 diffractometer (Rigaku, Japan) at a voltage of 40 kV and a current of 30 mA using Cu-Kα radiation with a scanning rate of 5°/min. The scanning scope of 2θ was ranged from 5 to 40° at ambient temperature.

2.3.3. Scanning electron microscopy

Micrographs of the samples were examined under a Quanta 200 scanning electron microscope (Philips-FEI Co., AMS, The Netherlands) with an accelerating voltage of 5 kv and at magnification of 500 (upper surfaces) and 1000 (cross-sections). The films were frozen in liquid nitrogen and snapped immediately to prepare the sample of upper surface and cross-sections. Prior to the observation, samples were coated with a thin gold layer.

2.3.4. Thermogravimetric analysis (TGA)

TGA of the samples was carried out on a TA Instruments TGA Q500 (TA Instruments, USA). The heating rate was 10 °C/min in the temperature range from 30 °C to 600 °C.

2.3.5. Light transmission and opacity measurements

Light transmission through the films (4 cm × 3 cm) was recorded in the range of 800–200 nm on an ultraviolet–visible (UV–vis) spectrophotometer (UV-2600, Shimadzu, Kyoto, Japan). Opacity of the films was calculated as below (Han & Floros, 1997):

$$\text{Opacity} = \text{Abs}_{600}/\text{X}$$

Where Abs_{600} is the value of absorbance at 600 nm and X is the film thickness (mm).

2.3.6. Thickness and tensile properties

Films thicknesses were measured with an ID-C112XBS micrometer (Mitutoyo Corp., Tokyo, Japan), and reported as the average from ten random points. Tensile tests were measured by using an auto tensile tester (XLW-PC, PARAM, Jinan, China) equipped with a 500 N load cell. Measurements were performed with a strain rate of 300 mm/min at 25 °C.

2.3.7. Oxygen permeability (OP)

A Perme OX2/230 (Labthink, Jinan, China) was utilized to measure OP values through films according to standard method ASTM D3985-05 (2002) at 23 °C and 0% RH. Each test consisted of three replicate measurements.

2.3.8. Water vapor permeability (WVP)

WVP values of the films were determined at 23 °C and 60% relative humidity by using a Mocon Permatran-W 3/61 (MOCON, MN, USA) according to GB/T 26253 (GB/T, 2010).

2.3.9. Antimicrobial activity

The disk inhibition zone assay was used to evaluate the antimicrobial activity of the films (Seydim & Sarikus, 2006). Film samples were aseptically cut into disc shapes with diameter of 0.8 cm and then placed on NB plates, which had been previously smeared with 200 μl of NA culture containing approximately 10^8 CFU/ml of bacteria. The plates were incubated at 37 °C for 12 h. The diameter of the inhibition zone was measured using a caliper to the nearest 0.05 cm. The reported inhibition zone values represent the average of all three samples.

2.3.10. Coating of bananas

Using a brush, we coated the entire surface of bananas with a film-forming CMC solution or with a solution of HTCC/CMC at specified mass ratio at 30 °C (Chen, Zhang, Zhao, & Chen, 2014). They were subsequently blow-dried for 30 min and stored at 30 °C and 40% relative humidity (RH). Photographs of the control bananas and coated bananas were taken at different times.

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