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Combined effects of two kinds of essential oils on physical, mechanical and structural properties of chitosan films

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ABSTRACT

The combined effects of plant essential oils (lemon, thyme, cinnamon) on the physical and structural properties of chitosan-based films were investigated. Results showed that the apparent viscosity and average particle size of lemon essential oils were significantly lower than that of the thyme and cinnamon essential oils. The combined use of two kinds of essential oils decreased the particle size and water vapor permeability compared with the use of a single essential oil. However, the combined use of two kinds of essential oils balanced the antibacterial properties of the single essential oil. SEM analysis showed that the oil droplets were homogenously distributed across the film. The emulsification was obviously observed in chitosan/lemon/cinnamon essential oils composite films due to the electrostatic interaction of limonene and cinnamaldehyde. This study revealed that an active chitosan film could be obtained by the combined use of two kinds of essential oils in the matrix, which might provide a new formulation option for developing antimicrobial film.

1. Introduction

One of the major challenges for food technologists of late has been the design of active food packaging. Active food packaging may carry antimicrobial agents in packaging systems, which may be more effective than applying antibacterial substances directly to the food surface due to providing continuous release of active additives from packaging materials (Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2009). Moreover, given the increasing health concerns of consumers, current packaging research has focused on the use of natural antimicrobial agents in edible packaging materials (Atarés, Bonilla, & Chiralt, 2010; Buonocore, Del Nobile, Panizza, Corbo, & Nicolais, 2003; Fisher & Phillips, 2008; Sánchez-González, Cháfer, Chiralt, & González-Martínez, 2010). Edible packaging materials usually consist of proteins, lipids and polysaccharides. Among the polysaccharides, chitosan has been studied extensively in the food industry due to its excellent film-forming, antimicrobial, physical and mechanical properties (No, Meyers, Prinyawiwatkul, & Xu, 2007; Ojagh, Rezaei, Razavi, & Hosseini, 2010; Park & Zhao, 2004; Sánchez-González et al., 2010; Zhong, Song, & Li, 2011).

Plant essential oils (EOs) are interesting natural antimicrobial agents to be incorporated into the edible films due to these plant extracts exhibit additional characteristics, such as antimicrobial and antioxidant effects (Atarés et al., 2010; Bagamboula, Uyttendaele, & Debevere, 2004; Fisher & Phillips, 2006; Pelissari, Grossmann, Yamashita, & Pineda, 2009). Moreover, according to the application of EOs, it is also important to evaluate their effects on the physical, optical and structural properties of the resulting film (Altiok, Altiok, & Tihminlioglu, 2010; Ojagh et al., 2010; Sánchez-González et al., 2010). Among the great variety of EOs, lemon, thyme and cinnamon EOs have gained greater acceptance amongst food technologists due to their better sensory evaluation and antimicrobial properties (Bagamboula et al., 2004; Fisher & Phillips, 2006; Viuda-Martos, Ruiz-Navajas, Fernández-López, & Perez-Álvarez, 2008). However, little information on the comparative effects of lemon, thyme and cinnamon EOs on the physical and structural properties of chitosan film is known.

In addition, in the cosmetics industry, the sensory flavor and application value of EOs have typically been improved by mixing several types of them together with the aid of surfactants. In food packaging, Ojagh et al. (2010) studied the addition of cinnamon EOs into chitosan films to protect against gram-positive and gram-negative bacteria by the combined use of Tween 80. Casariego et al. (2008) reported that surfactants such as Tween could also increase the wettability of the coating solutions and improve the

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adhesive capacity on food surface. Moreover, the water barrier and mechanical properties of films could be developed by incorporating EOs and hydrophilic surfactants (Chen, Kuo, & Lai, 2009; Sánchez-González, Vargas, González-Martínez, Chiralt, & Cháfer, 2009). However, there is little work concerning the combined effects of two kinds of EOs on the physical and structural properties of chitosan films with the emulsification of Tween.

Therefore, the purpose of the present work was to investigate the effects of the type and mixture of three EOs (lemon, thyme and cinnamon) on the antimicrobial, mechanical, optical and structural properties of chitosan-based films.

2. Materials and methods

2.1. Materials

Crab chitosan (deacetylated degree: 90.2%, viscosity: 62cps at 25 °C) was purchased from AK Biotech Ltd. (Shandong, China). Glycerol, Tween 20, acetic acid, magnesium nitrate ($Mg(NO_3)_2$) and anhydrous calcium chloride ($CaCl_2$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Lemon (*Citrus limonum*, limonene: 80.05%, β-pinene: 10.46%, citral: 4.31%), thymus vulgaris (*Thymus mongolicus*, thymol: 57.24%, p-cymene, 18.91%, carvacrol: 2.82%, linalool, 2.03%) and cinnamon branch (*Cinnamomum zeylanicum*, cinnamaldehyde: 61.17%, linalool: 6.47%, methoxycinnamaldehyde: 3.61%, caryephyllene: 3.47%) essential oil used in this study were provided by Shanghai Tiamay Aromatic Plant Sci. & Tech. Co. Ltd. (Shanghai, China). Nutrient broth medium was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *Escherichia coli* CMCC44102 and *Staphylococcus aureus* ATCC6538 were provided by the lab of Department of Food Science and Technology, Shanghai Jiao Tong University (Shanghai, China).

2.2. Film preparation

Chitosan (CH) solution (2%, w/v) was prepared by dispersing chitosan in an aqueous solution of glacial acetic acid (1%, v/v) at 25 °C and stirred with a magnetic stirrer (Shanghai Huxi Analysis Instrument Co., Ltd., Shanghai, China) for 4 h. After chitosan was completely dissolved, glycerol (0.6 g), Tween 20 (0.1 g) and EOs (1 mL single EO or blend EOs for each accounted 0.5 mL) were added into 100 mL chitosan solutions. All solutions were homogenized by using a rotor-stator homogenizer (IKA T25-Digital Ultra-Turrax, Staufen, Germany) at 13,500 rpm for 4 min. Finally, these film-forming solutions were vacuum degasified at room temperature (25 °C) with a vacuum pump for 1 h to remove air bubbles. Sample nomenclature was CH-X, X represents lemon (L), thyme (T) or cinnamon (C).

A casting method was used to obtain films. Film-forming solutions (200 mL) were cast over the leveled glass plates (25 × 25 cm) and dried at 25 ± 1 °C for 48 h. Then, the dried films were carefully peeled and stored for 48 h in desiccators containing $Mg(NO_3)_2$ saturated solutions (53% relative humidity) at 25 °C before next tests.

2.3. Rheological behavior of film solutions

The rheological behavior of film-forming solutions was analysed according to the method described by Sánchez-González et al. (2010) using a R/S plus rotational rheometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) with a fixed outer cylinder and rotating measuring bob. The radius of rotating cylinder was 12.50 mm, the length of the cylinder and the gap width was 50.00 mm and 2.00 mm, respectively. Rheological curves were obtained after 5 min stabilisation at 25 °C. The shear stress (σ) was measured as a function of shear rate (γ) from 0 to 512 s^{-1}, taking 5 min to reach the maximum shear rate and another 5 min to attain zero shear rate. Experiment data were fitted to the Ostwald de Waale model (Eq. (1)) in order to determine the consistency (k) and the flow behavior indexes (n). Apparent viscosities were calculated in 100 s^{-1}. The measurements were performed in triplicate at 25 °C.

$$\sigma = k\gamma^n \quad (1)$$

2.4. Particle size of film solutions

The particle size of the film solution was determined by means of dynamic light scattering (DLS) using a BI-90 plus particle size analyzer (Brookhaven Instruments Corporation, New York, USA) according to the method described previously by Li and Huang (2012). The light source was a solid state laser operated at the wavelength of 660 nm and the signals were detected by a high-sensitive avalanche photodiode detector. All samples were performed at 25 ± 1 °C with the detection angle of 90 °C and refractive index of 1.33. The autocorrelation functions, $G(\tau)$, were obtained by analyzing time-dependent signals with the Sigert relation (Stepanek, 1993). The measurements were performed in triplicate at 25 °C.

2.5. Antimicrobial tests

The agar diffusion method was used to determine the antibacterial effects of film solutions on bacterial strains. Filter paper discs (Whatman No. 1, 6 mm diameter) containing film solutions of 10 μL respectively were applied to the surface of agar plates that were previously seeded with 200 μL of bacterial cell suspensions. The concentrations of the *E. coli* and *S. aureus* suspensions were 4×10^7 CFU/mL and 6×10^7 CFU/mL, respectively. The plates were incubated at 37 °C for 24 h in the incubation chamber. The whole zone area was calculated then subtracted from the paper disc area and the difference in zone area was reported as the "zone of inhibition" (Seydim & Sarikus, 2006). The tests were performed in triplicate.

2.6. Water barrier properties of films

A hand-held micrometer (with an accuracy of 0.01 mm) was used to measure the thickness of the film. Six replications were performed for each sample at random positions. Water content of films was determined by drying in an oven at 105 °C for 24 h.

Water vapor permeability (WVP) was determined gravimetrically based on the method described by Talja, Helén, Roos, and Jouppila (2008) with some modifications. The films (60×60 mm^2) were sealed onto permeation cells (inner diameter: 42 mm, height: 25 mm) filled with granular ($\Phi < 2$ mm) anhydrous calcium chloride. The permeation cell was covered with a film sample attached with tape to guarantee that there is no leakage. The stagnant air gap under the films was less than 6 mm. The permeation cells were then placed in desiccators containing saturated NaCl solutions, providing 0–75% relative humidity gradients at 25 °C. The permeation cells weight was recorded when the changes were close to 0.001 g. WVP was calculated as follows:

$$WVP = \frac{mL}{At\Delta p} \quad (2)$$

Where m was the weight of water permeated through the film (g), L was the thickness of the film (m), A was the permeation area (m^2), t was the time of permeation (s), and Δp was water vapor pressure difference across the film (Pa). Five repetitions were performed for each film sample.