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Physicochemical properties, antimicrobial activity and oil release of fish gelatin films incorporated with cinnamon essential oil

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ABSTRACT

Fish skin gelatin films incorporated with various concentrations of cinnamon essential oil (CEO) were prepared and characterized. The results showed that tensile strength (TS), elongation at break (EAB), and water content (WC) of the gelatin based film decreased with the increasing concentrations of CEO, but water vapor permeability (WVP) increased. Addition of CEO improved light barrier property of the film. The Scanning electron microscope (SEM) showed that the heterogeneous surface and porous formation appeared in gelatin-CEO films. Fourier transform infrared spectroscopy analyses (FTIR–ATR) spectra indicated the interactions existed between gelatin and CEO. The gelatin-CEO films exhibited good inhibitory effects against the tested microorganisms (*Escherichia coli, Staphylococcus aureus*, *Aspergillus niger, Rhizopus oryzae*, and *Paecilomyces varioti*) and their antifungal activity seemed to be more effective than the resistance to bacterial growth. *In vitro* release studies showed an initial burst effect of CEO release and that subsequently slowed down at 40 °C, but the initial burst release was not obvious at 4 °C. The obtained results suggested that incorporation of CEO as a natural antimicrobial agent into gelatin film has potential for developing as active food packaging.

1. Introduction

In recent years, the number of food–borne diseases caused by pathogenic microorganisms has increased (Pelissari, Maria, Fabio, & Edgardo, 2009). During storage, some food-borne pathogenic microorganisms are prone to generate food spoilage and thus the demand for antimicrobial packaging containing efficient antimicrobial agents is increasing. Several studies have shown that packaging films incorporated with antimicrobial agents could effectively reduce the levels of food-borne microorganisms (Campos-Requena, Rivas, Perez, Figueroa, & Sanfuentes, 2015; Emiroğlu, Yemiş, Coşkun, & Candoğan, 2010). However, the recognition of potential toxicity of synthetic antimicrobials and health benefit encourage the quest for biodegradable packing materials containing natural antimicrobial agents. Therefore, research on developing new biodegradable packing materials containing natural antimicrobial compounds is a developing trend to increase shelf life for fresh and guarantee high quality of food products.

Protein is one of the main biopolymers for making edible packaging films (Ramos, Valdés, Beltrán, & Garrigós, 2016; Wang, Hu, Gao, Ye, & Wang, 2017). Protein film has excellent barrier properties against gas and volatile compounds, oils and UV light (Tongnuanchan, Benjakul, Prodpran, Pisuchpen, & Osako, 2016). Gelatin, obtained by denaturation and hydrolysis of collagen, is widely used in the manufacture of edible films due to excellent film forming ability, low gelling and melting point and biodegradability (Limpisophon, Tanaka, Weng, Abe, & Osako, 2009). Meanwhile, it is unique among hydrocolloids because its melting point is close to the body temperature, which is particularly significant in edible and pharmaceutical applications (Achet & He, 1995). However, the film just made from gelatin has poor antimicrobial properties.

Essential oils (EOs), which are hydrophobic and volatile compounds derived from plants, have shown potential bactericidal properties not only against food–poisoning bacteria but also against some common fungi and they have been gaining increasing attention within the food industry (Seydim & Sarikus, 2006; Wang *et al.*, 2015). Lemon, sage, and thyme EOs have been applied in cheese preservation (Gammariello, Di Giulio, Conte, & Del Nobile, 2008) and oregano EO was used to preserve fish muscle (Wu *et al.*, 2014). Meanwhile, EOs have been studied as bioactive additives in edible/biodegradable emulsified films (Atarés & Chiralt, 2016). The biogenic gelatin film incorporated with oregano and

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lavender EOs have been shown to inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* (Martucci, Gende, Neira, & Ruseckaite, 2015) and addition of *Ziziphora clinopodioides* EO into chitosan and gelatin films has been shown to improve antibacterial activity (Shahbazi, 2017). Cinnamon EO (CEO) has been found to have an effective antimicrobial activity and listed as "Generally Recognized as Safe-GRAS" by the Food and Drug Administration in 21 e-CFR (electronic Code of Federal Regulation) part 182.20 (Valero & Salmerón, 2003; Zhang, Li, Lv, Li, Kong, & Luo, 2017). Cinnamaldehyde, the major active component of CEO, has been shown to have broad-spectrum antimicrobial activity against bacteria, yeasts, and molds (Chen, Wu, McClementsd, Li, & Li, 2017). Therefore, CEO as natural bactericide could be an ideal choice to be added into films for the shelf life extension and quality improvement.

The aim of the present study was to develop gelatin films incorporated with CEO and characterize their properties, including mechanical strength, barrier, light transmission, microstructure, infrared spectrum, and antibacterial and antifungal activities. The *in vitro* release test of CEO from gelatin based films into corn oil was also characterized.

2. Materials and methods

2.1. Materials

CEO was bought from High Island Cosmetics Company (Guangzhou, China). Tween 80 and nutrient agar medium were purchased from Fu Chen Chemical Reagent Factory (Tianjin, China). The brain-heart infusion broth medium (BHI) was purchased from Ring Kay Microbial Technology Co., LTD (Guangdong, China). Glycerol was purchased from Sinopharm Chemical Reagent Co., LTD. The microorganisms were obtained from the College of Biological Science and Technology, Fuzhou University (Fujian, China).

2.2. Preparation of gelatin–CEO films

Gelatin was extracted from silver carp (*Hypophthalmichthys molitrix*) skins according to the procedure of Wu *et al.* (2013). After residual meat was removed, fish skin was washed thoroughly, cut into small pieces, soaked in 0.01 M NaOH containing 1% H_2O_2 with a skin/solution ratio of 1/20 (w/v) at 4 °C for 24 h with a gentle stirring. The solution was changed every 8 h for 3 times to remove non-collagenous proteins and pigments. The skin was subsequently washed with distilled water to neutral pH and defatted by soaking with 10% isopropanol (equal volume of the previous NaOH solution) at 4 °C for 4 h with a gentle stirring. Then, the treated fish skin was washed thoroughly with distilled water and swelled in 0.05 M acetic acid with an equal volume of the previous NaOH solution at 4 °C for 4 h with a gentle stirring, washed with distilled water to neutral pH, and subsequently incubated in distilled water with a half volume of acetic acid solution at 45 °C for 12 h with a continuous stirring to extract the gelatin. The mixture was centrifuged at 18,000*g*, 10 °C for 20 min and the supernatant was collected and freeze-dried. The dry matter was referred to as "gelatin".

The films were prepared using casting technique. Silver carp skin gelatin at a ratio of 4% (w/v) was dissolved in distilled water at 45 °C for 30 min to prepare film-forming solution (FFS). CEO (CEO/FFS = 0.5, 1, 2, 4, 6%, v/v) previously mixed with Tween-80 at 0.5% (v/v, based on FFS) were added into FFS. Glycerol as a plasticiser in 25% (w/w) based on gelatin weight was also added into FFS. The FFS was homogenized to make the CEO emulsify entirely at a speed of 5000 rpm for 10 min under an ice-bath condition with an Ultra-Turrax (IKA, Germany). The air bubbles in film solution were removed by ultrasound. The degassed film solution (10 mL) was cast into a plexiglass plate (120 mm × 80 mm × 1 mm) and dried at 22 ± 0.5 °C and 50 ± 2% relative humidity for 48 h. Then, the dried films were removed from plates and conditioned at 22 ± 0.5 °C and 50 ± 2% relative humidity in desiccators for at least 3 days before testing. The FFS without CEO was prepared in the same way.

2.3. Thickness

The thickness of the films was determined by using a micrometer caliper. Eight random locations around each film were measured for the average thickness determination.

2.4. Mechanical properties

The TA.XT–plus Texture Analyzer (Stable Micro System, UK) was used to determine tensile strength (TS) and elongation at break (EAB) with the ASTM standard method D 882 (ASTM, 2001). Each film was cut into 60 mm × 20 mm strips. The film samples were mounted between two grips with an initial grip separation of 5 cm and then pulled apart at a speed of 1 mm/s, respectively. TS and EAB were estimated in six samples from each type of film, of which the formulas are as (1) and (2), respectively.

$$\mathrm{TS(MPa)} = \mathrm{Fmax}/\mathrm{A} \quad (1)$$

Where Fmax is the maximum load (N) needed for snapping the sample apart, A is the initial cross–sectional area (m2) of the sample.

$$\mathrm{EAB}(\%) = (\Delta \mathrm{L}/\mathrm{L}) \times 100 \quad (2)$$

Where L is the original length of the film, ΔL is the stretched length when the film breaks.

2.5. Water content

Film samples were first weighed (W_1), and then dried at 105 °C for one day and weighed again (W_2). Water content (WC) was determined as the percentage of weight reduction of initial film during drying and reported on a wet basis. Triplicate tests of WC were conducted for each type of film, and an average was taken as the result. The WC of the films was calculated using the following equation:

$$\mathrm{WC}(\%) = (W_1 - W_2)/W_1 \times 100 \quad (3)$$

2.6. Water vapor permeability

The water vapor permeability (WVP) was measured gravimetrically using the wet bottle method (Wu *et al.*, 2013). A 14 mL of penicillin bottle was filled with distilled water (10 mL) to a height of 12 mm from the top edge and sealed by the test film (1.539 cm^2 of film area). The sealed bottle was initially weighed and placed in a controlled temperature and RH chamber with silica gel. Then the weight of the sealed bottle was measured at 12 h intervals for 3 days using a digital balance. Triplicate tests of WVP were conducted for each type of film. The WVP value was calculated as following:

$$\mathrm{WVP}\left(\mathrm{g\ m^{-1}Pa^{-1}s^{-1}}\right) = (\mathrm{W} \times \mathrm{X})/(\mathrm{A} \times \mathrm{t} \times \Delta \mathrm{P}) \quad (4)$$

Where W is the weight lost of the bottle (g), X is the thickness of film (m), A is the measuring area of exposed film (m^2), t is the time (s), and ΔP is partial vapor pressure difference of the atmosphere with silica gel and pure water.

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