Development and characterization of novel antimicrobial bilayer films based on Polylactic acid (PLA)/Pickering emulsions

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ABSTRACT

Biodegradable food packaging is sustainable and has a great application prospect. PLA is a promising alternative for petroleum-derived polymers. However, PLA packaging suffers from poor barrier properties compared with petroleum-derived ones. To address this issue, we designed bilayer films based on PLA and Pickering emulsions. The formed bilayer films were compact and uniform and double layers were combined firmly. This strategy enhanced mechanical resistance, ductility and moisture barrier of Pickering emulsion films, and concomitantly enhanced the oxygen barrier for PLA films. Thymol loadings in Pickering emulsion layer endowed them with antimicrobial and antioxidant activity. The release profile of thymol was well fitted with Fick's second law. The antimicrobial activity of the films depended on film types, and Pickering emulsion layer presented larger inhibition zone than PLA layer, hinting that the films possessed directional releasing role. This study opens a promising route to fabricate bilayer architecture creating synergism of each layer.

1. Introduction

Tremendous attention has been attracted to reduce the reliance on fossil fuels-derived polymers to face the environmental challenges caused by the plastic wastes, in view that the plastic amount have been forecasted to arrive at 33 billion tons in 2050 (Goh et al., 2016; Rochman et al., 2013). Biopolymers are from renewable resources and biodegradable, thus own an environmental advantage over fossil fuels-derived polymers (Gross & Kalra, 2002). In many choices, poly (lactic acid) (PLA) has been regarded as a promising substitute for petroleum-derived polymers. PLA is derived from regenerative resources, and is biodegradable and environmentally friendly (Drumright, Gruber, & Henton, 2000; Gross & Kalra, 2002). Furthermore, it is safe as food packaging materials (Conn et al., 1995; Ramot et al., 2016). Nonetheless, the intrinsic barrier properties of PLA are needed to be addressed to make it competitive with petroleum-derived polymers (Goh et al., 2016). Fabrication of bilayer and/or trilayer films is a promising strategy to enhance physical performance of films. However, the direct formation of multilayer films tends to delaminate over time, and develop crack or pinholes. PLA and biopolymers (e.g., starch, cellulose) had poor interfacial adhesion, and plasma treatment was usually used to modify the surface properties of PLA films, such as wettability and

surface energy, enabling the deposition of thin coatings on the polymer surface as well as development of multilayer films (Meriçer et al., 2016).

Pickering emulsion is a kind of solid particle-stabilized emulsion. Unlike surfactants, solid particles are apt to irreversibly adsorb toward the surface of oil droplets and establish firm, rigid and robust interface layer architecture thereby, and high resistance against coalescence is a major advantage of Pickering emulsions (Binks, 2002). The stabilization phenomena of Pickering emulsions depend on the accumulation and interaction of solid particles at the boundary of oil and water phase in a form of packed and firm physical barrier, as a result, prevents Pickering emulsion coalescence and demulsification (Binks, 2002). Therefore, engineering interfacial structure of emulsions via the facile Pickering approach is a potential route to enhance of the stability of film-forming emulsions (FFEs), concomitantly improve the performance of the formed emulsion films. In a previous work, we found that Pickering emulsion-based FFEs were extremely stable, evidenced by the similar droplets size and size distribution in the film matrix and FFEs, Pickering droplets were even-distributed over their 3D network matrix of the films (Shi et al., 2016). In fact, conventional emulsion films usually appeared a density gradient of lipid droplets along longitudinal section of the films. Therefore, the distribution of lipid droplets was

heterogeneous within 3D network matrix and oil phase was rich in the near-surface region of the films, possibly due to the creaming and/or coalescence of the droplets in FFEs during the casting (Wang et al., 2013).

PLA film and Pickering emulsion film had their own advantage. The aim of this work was to fabricate PLA/Pickering emulsion bilayer films which had the complementary advantage of each simple film. Thymol possesses wide antibacterial effect and has excellent antioxidant activity (Ramos, Beltrán, Peltzer, Valente, & Garrigós, 2014), and it is generally recognized as safe (GRAS). Thus, this work also attempted to develop active bilayer films with Pickering emulsion layer as thymol vehicle. Antibacterial properties, antioxidant capacity, and thymol release dynamics of bilayer films were evaluated. Water vapor barrier, microstructure, and mechanical properties of the films were investigated. In addition, the thymol release dynamics was fitted to Fick's second law and the possible structure-function relationship of the films was discussed.

2. Materials and methods

2.1. Materials

Poly (L-lactic acid) (PLA 4032D) was purchased from Nature Works LLC (Blair, NE, USA). Zein (product Z3625) and DPPH (2, 2-Diphenyl-1-picrylhydrazyl) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Maize germ oil (0.87 g/mL) was obtained from local supermarket (Guangzhou, China). Polyethylene glycol (PEG 2000), chloroform, glycerol, and other chemicals used were of analytical grade.

2.2. Preparation of zein/chitosan complex particles (ZCCPs)

ZCCPs were prepared according to the procedure reported by Shi et al. (2016), with slight modifications. In brief, 100 mL of zein solution (2.5% in 80% aqueous ethanol) was slowly (2 min) dropped into 250 mL chitosan solution (0.05% in 1% acetic acid solution) under shearing (5000 rpm) using an Ultra-turrax T25 homogenizer (IKA, Germany). After another 2 min of shearing, the suspension was concentrated via rotary evaporation and then centrifuged (4000 rpm) to remove the insoluble aggregates to obtain stable ZCCPs suspension with particle concentration of approximately 4% by weight.

2.3. Preparation of PLA film

PLA film was prepared according to the procedure reported by Baiardo, Frisoni, Scandola, & Wintermantel (2003) and Jamshidian, Tehrany, Imran, & Desobry (2012), with slight modifications. Both PLA and polyethylene glycol (PEG) (the weight ratio PEG to PLA is 1/9) was dissolved in chloroform with the solute concentration 3.5% (w/v). The mixture was magnetically stirred for an hour. Twenty-three milliliters of solution was poured into glass plate (10cm × 17 cm) coated with polytetrafluoroethylene (Teflon) film. The glass plate was covered by polyethylene film having several holes to control evaporation rate and the layer of PLA film was formed which took approximately 3 h at room temperature. PLA film was made by the above methods using 46 mL of solution and the time of drying process lasted more than 5 h.

2.4. Preparation of pickering emulsion film

Maize germ oil (2 mL) was mixed with ZCCPs suspension (18 mL) and then the mixture was sheared (5000 r/min) for 5 min using an Ultra-Turrax T10 homogenizer to prepare Pickering emulsion. The Pickering emulsion was blended with 20 mL chitosan solution (2%, w/v; glycerol/chitosan, 1/5) and the blends were further homogenized within ultrasound generator (Scientz-IID, China) equipped with an ultrasonic probe (10 mm in diameter) to prepare film-forming solution

(FFS). The ultrasonic power used was 370 W and the samples were treated for 5 min during which ultrasound time and interval time were 3 s. An aliquot (15 mL) of FFS was poured onto the PLA surface, and then bilayer film was obtained after drying 24 h in a thermostatic chamber in which the temperature and relative humidity (RH) was maintained at 25 ± 1 °C and 50 ± 5%, respectively. An aliquot (40 mL) of FFS was poured into the glass plate (10cm × 17 cm) coated with polyethylene film to prepare Pickering emulsion film. All the films were stored in the condition of 25 °C, 50%RH.

2.5. Particle size and size distribution of ZCCPs and pickering emulsions

The particle size and size distribution of ZCCPs was measured by dynamic light scattering (DLS) using Zetasizer Nano S90 (Malvern Instruments Ltd, UK) after appropriate dilution with Milli-Q water. The particle size and size distribution of Pickering emulsions was measured using Mastersizer 3000 (Malvern Instruments, UK) according to the procedure reported by Wang, Hu, Yin, Yang, Lai, & Wang (2015).

2.6. Film thickness measurement

Film thickness was measured with digital micrometer and the resolution is 0.001 mm according to the procedure reported by Wang et al. (2013). Films thickness for tensile strength calculations were measured along the length of film strips, whereas films thicknesses at five random points of circular film disks were measured for water vapor permeability (WVP) and oxygen permeability (OP) calculations.

2.7. Mechanical properties measurements

Tensile properties, i.e., elastic modulus (EM), tensile strength (TS), and elongation at break (EAB) of films were evaluated according to ASTM protocol (ASTM, 2001). Specimens of 2.5 × 10 cm rectangular strips were used for tensile testing. Film strips were preconditioned at 25 °C and 50 ± 3% RH at least 2 days prior to analysis. The initial interval and crosshead speed were set as 50 mm and 1 mm/s, respectively. The maximum load used in tensile measurements is 100N. For each sample, at least eight specimens were tested.

2.8. Water vapor permeability (WVP)

WVP of the films was analyzed by A TSY- H1H WVP Analyzer (Jinan, Shandong, China) via the ASTM E96 procedure (ASTM, 1995). The detailed procedure was described by Shi et al. (2016).

2.9. Oxygen permeability measurements

Oxygen permeability (OP) of films was measured using a VAC-V1 Oxygen Permeation Analyzer (ShanDong, China) according to the ASTM D1434 procedure (ASTM, 2009). The films were cut into circular with diameter 9.7 cm after storing at least 2 days in the circumstance of 25 °C and 50% RH. Film specimen was fixed in the oxygen permeation device in which the test area of specimen is 38.46 cm^2. Evacuation time was set to 8 h, and the oxygen pressure was set to 0.5 MPa.

2.10. Transmittance and opacity of films

The UV − visible spectra of the films were recorded from 200 to 800 nm in a UV-2300 spectrophotometer (TianMei Techcomp Limited., Shanghai, China).

2.11. Scanning electron microscopy (SEM)

Scanning electron microscope (EVO18, Germany) was used to observe and analyze the microstructure of films. With upper surface outward, films specimens were adhered to the surface and side face of