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Physical and antimicrobial properties of chitosan films incorporated with lauric arginate, cinnamon oil, and ethylenediaminetetraacetate

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ABSTRACT

Lauric arginate (LAE) and cinnamon oil (CO) combination results in synergistic antimicrobial effect on Gram-positive bacteria but antagonistic effect on Gram-negative bacteria. We recently observed that ethylenediaminetetraacetate (EDTA) enhanced the activity of LAE and overcame the antagonistic effect of LAE-CO combination. The objective of this work was to study physical and antimicrobial properties of chitosan films with LAE, CO, and EDTA. A significant increase in the thickness was detected after incorporating antimicrobials in chitosan films. The yellowness of films increased, while water solubility decreased as the concentration of CO increased. Water vapor permeability of films increased with the addition of the antimicrobials. Incorporation of antimicrobials in chitosan films lowered the tensile strength but did not affect elongation%. CO content in the films was significantly higher with at a higher LAE content. Much larger inhibition zones of film discs with antimicrobials against foodborne pathogens were detected compared to those of films with chitosan only. Addition of EDTA enhanced the antimicrobial activity of films with LAE, while binding by CO reduced the amount LAE diffusing from films to the media to inhibit bacteria. Overall, these novel antimicrobial films with LAE, CO, and EDTA showed potential to improve the safety of food products.

1. Introduction

Antimicrobial films and coatings are potential intervention strategies to control foodborne pathogens contaminating food products (Chen, Jin, Gurtler, Geveke, & Fan, 2012; Higueras, López-Carballo, Hernández-Muñoz, Gavara, & Rollini, 2013). Natural antimicrobials have received particular interests because they are perceived by consumers to be safe and healthy. Examples of natural antimicrobials include essential oils (EOs) such as eugenol, cinnamon oil (CO), and thyme oil which have shown great antimicrobial activities (Chen, Zhang, & Zhong, 2015; Ma, Davidson, & Zhong, 2013; Pan, Chen, Davidson, & Zhong, 2014; Xue & Zhong, 2014). Therefore, antimicrobial films/coatings incorporated with EOs have been investigated by many researchers (Hosseini, Razavi, & Mousavi, 2009; Zivanovic, Chi, & Draughon, 2005). A coating solution consisting of 2 g/100 mL chitosan and 1.5 mL/100 mL CO maintained the total viable aerobic bacterial counts on rainbow trout fillets below 6 $\log_{10}$ CFU/g during 16-day storage at 4 °C (Ojagh, Rezaei, Razavi, & Hosseini, 2010b). Coatings with 1 g/100 g chitosan and 3 g/100 g lemon oil significantly reduced the fungal decay percentage of strawberries stored at 5 °C for 3 days, when compared to that of uncoated strawberries (Perdones, Sánchez-González, Chiralt, & Vargas, 2012).

Lauric arginate (ethyl-Nα-lauroyl-L-arginine ethyl ester monohydrochloride; LAE) is another effective antimicrobial that has been approved by the United States Food and Drug Administration as a generally-recognized-as-safe food additive (USDA, 2005). LAE is a cationic surfactant derived from lauric acid, L-arginine and ethanol, has a low toxicity (Ruckman, Rocabayera, Borzelleca, & Sandusky, 2004), and is highly efficacious in inhibiting foodborne pathogens (Ma et al., 2013). The minimum inhibitory concentration (MIC) of LAE against ca. 6 log CFU/mL *Listeria monocytogenes* in tryptic soy broth (TSB) at 32 °C was determined to be 11.8 μg/L (Ma et al., 2013). Applying a solution with 22 μL/L LAE on the surface of frankfurters resulted in more than 1 log CFU/cm^2 reduction of *L. monocytogenes* within 12 h at 4 °C (Martin et al., 2009). Studies on antimicrobial films with LAE however are scarce.

Because LAE has a bitter taste and EOs have strong aromas, combinations of these antimicrobials may lower the concentrations

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of individual antimicrobials if they have synergistic activities. We recently showed that the combination of LAE and EOs had the synergistic activity inhibiting Gram-positive *L. monocytogenes* but had the antagonistic effect against Gram-negative *Escherichia coli* O157:H7 and *Salmonella* Enteritidis (Ma et al., 2013). In our separate study to be submitted elsewhere, 500 μg/L EDTA resulted in much enhanced activity of 5 μg/L LAE alone and its mixture with 200 μg/L cinnamon oil (CO) against both Gram-positive *L. monocytogenes* Scott A and Gram-negative *E. coli* O157:H7 and *S.* Enteritidis. EDTA is a safe and economical additive that chelates divalent cations (Ca^{2+} and Mg^{2+}) important to the structures of outer membranes of Gram-negative bacteria (Vaara, 1992). As a result, the activity of several antimicrobials is enhanced by EDTA (Branen & Davidson, 2004), and the same mechanism may overcome the antagonistic activity of LAE-EO combination. Therefore, it is possible to improve the antimicrobial activity of films with LAE only or with additional EO by including EDTA in film-forming mixtures.

The objective of the present study was to evaluate physical and antimicrobial properties of cast chitosan films incorporated with LAE, CO, and EDTA. Chitosan was studied as the film-forming biopolymer because it has excellent properties to form films with good mechanical properties (Elsabee & Abdou, 2013). Additionally, chitosan itself has antibacterial and antifungal activities (Kim, Thomas, Lee, & Park, 2003; Tsai, Su, Chen, & Pan, 2002).

2. Materials and methods

2.1. Materials

Chitosan (low molecular weight, 75–85% deacetylated), CO, and EDTA were purchased from Sigma–Aldrich Corp. (St. Louis, MO). The commercial LAE with a name of CytoGuard LA was kindly provided by A&B Ingredients (Fairfield, NJ). The product contained 10 g/100 g LAE and 90 g/100 g propylene glycol. Acetic acid and TSB were procured from Thermo Fisher Scientific, Inc. (Waltham, MA).

2.2. Film preparation

The chitosan stock solution was prepared by dissolving 2 g/100 g chitosan powder in an aqueous solution with 1 g/100 g acetic acid and stirring overnight on a magnetic stir plate at a low speed. The impurities were removed by filtering the solution through a microcloth (Calbiochem-Novabiochem Corp., San Diego, CA). LAE, EDTA, and CO were then directly added into the chitosan stock solution by mixing on a magnetic stir plate at room temperature (21 °C) until visually homogeneous. The final 100 g film-forming mixtures after supplementing deionized water contained 1 g chitosan, 0.5 g acetic acid, 0, 0.1 or 0.2 g LAE, 0 or 0.25 g EDTA, and 0, 0.5, or 1 g CO. Films were prepared by casting 30 g film-forming mixtures on 17.8 cm × 17.8 cm glass plates and drying at ambient conditions (21 °C) for 24 h. After peeling, films were conditioned at 57 g/100 g relative humidity (RH) controlled by a saturated sodium bromide solution in a desiccator for 48 h at 21 °C before characterizations. Films prepared with 1 g/100 g chitosan and 0.5 g/100 g acetic acid were treated as the control.

2.3. Physical and mechanical properties of films

2.3.1. Thickness

A digital microcaliper (Mitutoyo Corp., Kawasaki, Japan) was used to measure the thickness of chitosan films. The microcaliper had a precision of 0.001 mm. Twelve locations on various regimes of films were measured for each film and means and standard deviations were reported.

2.3.2. Color

Lightness (L) and chromaticity parameters a (red-green) and b (yellow-blue) in the Hunter Lab scale were measured in triplicate using a MiniScan XE Plus Hunter colorimeter (Hunter Associates Laboratory, Inc., Reston, VA) for each film. Color differences (ΔE) based on the standard white plate were calculated using Eq. (1) (Hosseini et al., 2009).

$$\Delta E = \sqrt{(a^* - a)^2 + (b^* - b)^2 + (L^* - L)^2} \quad (1)$$

where a, b, and L are the color parameter values of the film, and a^* (−1.11), b^* (0.57), and L^* (93.82) are the color parameter values of the standard white plate.

2.3.3. Moisture content and water solubility

To determine the moisture content and water solubility of films, 2 cm × 2 cm film squares were prepared and weighed (w_0). Film squares were dried at 60 °C in an oven for 24 h to a constant mass (Jiménez, Fabra, Talens, & Chiralt, 2012). After cooling to room temperature in a desiccator filled with anhydrous calcium chloride, film squares were weighed again (w_1). Moisture content was then calculated based on Eq. (2). Water solubility of films was measured by immersing the film squares into deionized water for 2 h at room temperature. After removing free water, film discs were put into an oven and dried at 60 °C for 24 h. Total solids mass of film discs was recorded after cooling to room temperature in a desiccator (w_3). Water solubility was calculated based on Eq. (3) (Rotta et al., 2009). Three film replicates prepared from each formulation were tested.

$$Moisture\left(\frac{g}{100g}\right) = \frac{w_0 - w_1}{w_1} \times 100 \quad (2)$$

$$Water\ solubility\left(\frac{g}{100g}\right) = \left(1 - \frac{w_3}{w_0 \times (100\% - moisture\ content)}\right) \times 100 \quad (3)$$

2.3.4. Water vapor permeability (WVP)

The WVP of films was measured using Fisher/Payne permeability cups with an opening area of 9.61 cm^2 (Fisher Scientific, Pittsburgh, PA). Films were sealed on cups which were pre-filled with 5.0 g deionized water (Zivanovic et al., 2005) and the cups were then placed in a desiccator with 57 g/100 g RH controlled by a saturated sodium bromide solution at room temperature (21 °C). The cup mass was measured with a precision of 0.0001 g every hour for up to 8 h. Water vapor permeation ratio (WVPR) was calculated based on the mass changes (M) over time (T) and effective film area (A) according to Eq. (4), while WVP was calculated using Eq. (5) (Pelissari, Grossmann, Yamashita, & Pineda, 2009). Measurements were performed in triplicate.

$$WVPR = \frac{M}{T \times A} \quad (4)$$

$$WVP = \frac{WVPR \times t}{sp \times (RH_1 - RH_2)} \quad (5)$$

where t is the thickness of films, RH_1 *and* RH_2 are the RH inside (100 g/100 g) and outside (57 g/100 g) the cup, and sp is the water vapor saturation pressure at the test temperature (Pa).

2.3.5. Tensile strength and elongation

A TA.XTplus Texture Analyzer (Texture Technologies Corp.,