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Physical, mechanical, and antimicrobial properties of chitosan films with microemulsions of cinnamon bark oil and soybean oil

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ABSTRACT

Antimicrobial films prepared with essential oil emulsions often have a high degree of opacity. This may be prevented using transparent microemulsions. The objective of this study was to characterize physical, mechanical, and antimicrobial properties of films prepared from mixtures with 1% w/w chitosan solution and microemulsions containing 1:0, 2:1, and 4:1 mass ratios of cinnamon bark oil (CBO) (1, 2 and 3% w/w) and soybean oil. Changes in solvent polarity after mixing chitosan solution and microemulsions increased droplet dimension from <30 nm of microemulsions to >88 nm of the film–forming mixtures and induced different extents of coalescence after film formation. Despite these physical changes, films prepared from microemulsions were transparent and had low opacity. The incorporation of microemulsions increased the thickness and water vapor permeability of films and significantly reduced the moisture content and swelling ratio. The retention of CBO was improved for films prepared from microemulsions with 2 and 3% CBO immediately following film formation and after ambient storage, when compared to control films prepared with emulsions with less Tween 80. Large zones of inhibition against foodborne pathogens were observed for film discs prepared with 2 and 3% CBO. These characteristics show the potential of using microemulsions as an easy approach to incorporate EOs in biopolymer antimicrobial films to improve microbiological safety and film transparency.

1. Introduction

Fresh produce is perishable and is sometimes associated with outbreaks of foodborne illnesses due to contamination by microbial pathogens (CDC, 2012a, 2012b). Many strategies to improve the microbiological safety and quality of fresh produce have been studied, including films or coatings with and without antimicrobials. Polysaccharides (Perdones, Vargas, Atarés, & Chiralt, 2014; Zivanovic, Chi, & Draughon, 2005), proteins (Gómez-Estaca, Montero, & Gómez-Guillén, 2014; Moditsi, Lazaridou, Moschakis, & Biliaderis, 2014) and lipids (Arcan & Yemenicioğlu, 2013) have been utilized as edible film–forming or coating materials. Chitosan, a copolymer consisting of β-(1–4)-2-acetamido-D-glucose and β-(1–4)-2-amino-D-glucose units, is an excellent film–forming material derived from chitin by N-deacetylation (Domard & Domard, 2001; Elsabee & Abdou, 2013). Chitosan films have good mechanical properties and a selective permeability (higher permeability to CO_2 and lower to O_2) (Despond, Espuche, & Domard, 2001; Elsabee et al., 2013). In addition, chitosan itself has antibacterial and antifungal activity (Kim, Thomas, Lee, & Park, 2003; Tsai, Su, Chen, & Pan, 2002). Thus, coating with chitosan may be a good strategy to improve the microbiological safety and quality of fresh produce (Sangsuwan, Rattanapanone, & Rachtanapun, 2008).

Various antimicrobials have been incorporated into films and plant essential oils (EOs) are frequently studied due to their broad antimicrobial activity (Chen, Zhang, & Zhong, 2014; Ma, Davidson, & Zhong, 2013). Incorporating EOs into chitosan films has been shown to enhance antimicrobial activity and lower water vapor permeability (WVP) (Ojagh, Rezaei, Razavi, & Hosseini, 2010; Pereda, Amica, & Marcovich, 2012; Zivanovic et al., 2005). However, adding EOs into chitosan films can also increase the opacity thus affecting the appearance of products (Hosseini, Razavi, & Mousavi, 2009; Pereda et al., 2012). This largely results from the low-water solubility of EOs that form particulate structures in the film to scatter visible light. In addition, the volatile nature of EOs

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causes their significant loss during film formation and storage (Chi, Zivanovic, & Penfield, 2006). Much work is still needed to improve the properties of antimicrobial films/coatings with EOs.

Colloidal systems are used to improve various functional properties of oil and water mixtures. Microemulsions are thermodynamically stable isotropic mixtures of water, oil, surfactants, and co-surfactants (Danielsson & Lindman, 1981). Microemulsions are transparent because their droplets are from 1 to 100 nm, typically 10–50 nm (Moulik & Paul, 1998; Slomkowski et al., 2011). The interfacial tension in microemulsions is very low, which enables their easy preparation without using high mechanical energy as in conventional emulsification (Klossek, Marcus, Touraud, & Kunz, 2014). Therefore, transparent films may be obtained by incorporating EO microemulsions in a biopolymer matrix. Furthermore, many fresh produce products, such as cantaloupes, have irregular and rough surfaces and entrapment of bacteria in the cavities on the produce surface can reduce or eliminate the effectiveness of antimicrobial films and coatings. This was demonstrated in a study on the influence of surface roughness of fresh produce on the adhesion rate of *Escherichia coli* O157:H7 (Wang, Feng, Liang, Luo, & Malyarchuk, 2009). A positive linear correlation was found between adhesion rate of the bacterium and surface roughness but a negative correlation existed between the surface roughness and inactivation efficacy by acidified electrolyzed water and peroxyacetic acid. In another study, the improved inactivation of *E. coli* O157:H7 on spinach leaves was reported after adding the surfactant, sucrose monolaurate, to a sodium hypochlorite wash solution (Xiao et al., 2011). This study illustrated that surfactants can lower the solid/liquid interfacial tension to facilitate access of antimicrobials to bacteria which are protected by the heterogeneous structures of fresh produce. Because surfactants are a part of microemulsions, coatings prepared from EO microemulsions may have the potential to enhance antimicrobial activity. Additionally, microemulsions can be formulated to dissolve long-chain triacylglycerols such as soybean oil (SBO) that may change evaporation properties of volatile compounds (Kim, Wu, Kubota, & Kobayashi, 1995) and film properties.

In a previous study (Ma & Zhong, 2015), we formulated microemulsions with an oil phase consisting of various mass ratios of cinnamon bark oil (CBO) and SBO using polysorbate 80 (Tween™ 80) as the surfactant and an equal mass of water and propylene glycol (PG) as the polar phase. The objective of the present work was to characterize physical, mechanical, and antimicrobial properties of films cast from mixtures of chitosan solution and the microemulsions formulated with various mass ratios of CBO and SBO.

2. Materials and methods

2.1. Materials

Low molecular weight chitosan (75–85% deacetylated) and CBO were purchased from Sigma–Aldrich Corp. (St. Louis, MO). PG, SBO, glycerol, acetic acid, tryptic soy broth (TSB) and Tween™ 80 were purchased from Thermo Fisher Scientific, Inc. (Waltham, MA).

2.2. Preparation of microemulsions

Microemulsions were prepared by simple mixing as previously described (Ma & Zhong, 2015). Microemulsions contained Tween™ 80, a polar phase with equal mass of PG and water, and an oil phase comprised of CBO and SBO at mass ratios of 1:0, 2:1, or 4:1 (abbreviated as microemulsion 1:0, 2:1, and 4:1, hereafter). Specific compositions of microemulsions are listed in Table 1. Tween™ 80, the oil phase and the polar phase were added together and mixed by hand shaking until a transparent appearance with no further visible changes.

2.3. Film preparation

A chitosan stock solution was prepared at 2% w/w in 1% w/w acetic acid solution. The impurities were removed by filtering the solution through a microcloth (Calbiochem–Novabiochem Corp., San Diego, CA). Microemulsions were mixed with the 2% w/w chitosan stock solution, glycerol (at 20% mass of chitosan), and deionized water to final CBO concentrations of 1, 2, and 3% w/w. The final concentrations of chitosan and acetic acid were 1% and 0.5% w/w, respectively. Films were prepared by casting 30 g of the mixtures on 17.8 cm × 17.8 cm glass plates and drying at ambient conditions (21 °C) for 24 h. Unless stated otherwise, the dried films were peeled and conditioned for about 48 h at room temperature in a desiccator with 57% relative humidity (RH) controlled by a saturated sodium bromide solution prior to physical characterizations. Films without microemulsions were prepared at the same chitosan concentration as a control.

2.4. Hydrodynamic diameters (D_h) of film–forming mixtures

The D_h of fresh film–forming mixtures was measured using dynamic light scattering. A Delsa Nano analyzer (Beckman Coulter, Atlanta, GA) with a scattering angle of 165° was used. Measurements were done in triplicate for each sample.

2.5. Viscosity of film–forming mixtures

The viscosities of film–forming mixtures were measured using an AR2000 rheometer (TA Instruments, Inc., New Castle, DE). About 20 mL of each sample was loaded into a bob–cup geometry with the outer diameter of bob and the inner diameter of cup being 28 and 30 mm, respectively. Shear rate ramps were conducted at a shear rate range of 0.1–100 s^{-1} at 25 °C, and each sample was sheared for 1 min. Each test was repeated at least once.

2.6. Physical and mechanical properties of films

2.6.1. Thickness

The thickness of films was measured using a digital microcaliper (Mitutoyo Corp., Kawasaki, Japan) with 0.001 mm precision. A total of 12 points were measured for each film. Means from two film replicates were reported.

2.6.2. Color and opacity

The color and opacity of films were measured using a MiniScan XE Plus Hunter colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). The color of films was measured for lightness (L) and chromaticity parameters a (red–green) and b (yellow–blue) in the Hunter Lab scale. Color measurements were performed over the standard white tile. Opacity was measured over the standard white tile and black glass. For each treatment, two film replicates were measured, each tested in triplicate.

2.6.3. Moisture content and swelling ratio

To determine the moisture content (Eq. (1)) and swelling ratio (Eq. (2)) of films, films were cut into 2 × 2 cm squares and weighed (w_1). Moisture contents of films were determined by drying the films at 60 °C for 24 h and weighing after cooling to room temperature in a desiccator filled with anhydrous calcium chloride (w_2). Swelling ratio was measured by immersing the film squares into deionized water for 24 h. Wet samples were tapped with filter paper to remove free water, followed by weighing the wet films