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Improving functional properties of chitosan films as active food packaging by incorporating with propolis

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ABSTRACT

Chitosan films containing 0, 2.5, 5, 10 and 20% w/w propolis extract (PE), high in polyphenols, were developed. The films' tensile strength, elongation at break, total phenolic content and antioxidant activity increased, while water vapor permeability and oxygen permeability decreased with increasing propolis concentration. Increasing PE concentration resulted in deeper orange color films, as compared to light yellow control films. The ability of the films to inhibit *Staphylococcus aureus, Salmonella* Enteritidis, *Escherichia coli* and *Pseudomonas aeruginosa* was determined using agar diffusion technique. Chitosan films containing PE could inhibit all tested bacteria on contact surface underneath the film discs. Changes in the Fourier Transform Infrared spectra of the films were observed when PE was incorporated, suggesting some interactions occurred between chitosan and propolis polyphenols. The characterization of mechanical properties, oxygen and moisture barrier, and antioxidant and antimicrobial activities revealed the benefits of adding PE into chitosan films and the potential of using the developed film as active food packaging.

1. Introduction

Recently, research and developments in active food packaging have focused on bio-based functional packaging materials incorporating natural active compounds and ingredients (Leceta, Guerrero, & de la Caba, 2013; van den Broek, Knoop, Kappen, & Boeriu, 2015; Madureira, Pereira, & Pintado, 2015). Chitosan is a functional biopolymer with intrinsic antimicrobial and antioxidant properties and consequently, it has high potential to be used as an alternative biodegradable active food package (Fernandez-Saiz, Lagaron, & Ocio, 2009; Guoa et al., 2015; van den Broek et al., 2015). Bio-based packaging materials with antioxidant and antimicrobial properties have become popular since oxidation and microbial contamination are major problems affecting food quality and safety. Many studies have been conducted on the utilization of plant polyphenols as alternatives to synthetic antimicrobial and antioxidant agents (Evrendilek, 2015; Lores, Pájaro, Álvarez-Casas, Domínguez, & García-Jares, 2015; Madureira et al., 2015; Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2015; Siripatrawan, 2016).

Propolis, the natural resinous substance collected by honeybees from various plant sources, is considered a good source of natural antioxidants and antibacterials (Bankova, 2005). Propolis contains a variety of chemical compounds such as polyphenols (flavonoid aglycones, phenolic acids and their esters, phenolic aldehydes, alcohols and ketones), sesquiterpene quinines, coumarins, amino acids and inorganic compounds (Bankova, 2005; Falcão et al., 2010). Flavonoids (flavones, flavonols and flavonones), aromatic acids and phenolic compounds are the most important active constituents of propolis and appear to be the principal components responsible for the biological activities of propolis samples (Silici & Kutluca, 2005). Propolis has been reported to possess various biological activities, such as antibacterial, antiviral, antitumor, anti-inflammatory, anticancer, antifungal, and antitumoral properties (Falcão et al., 2010). The antimicrobial effects of propolis against Gram-positive (*Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus*) and Gram-negative (*Salmonella* Typhimurium, *Escherichia coli* and *Pseudomonas fluorescence*) bacteria have been reported (Silici & Kutluca, 2005; Siripatrawan, Vitchayakitti, & Sanguandeekul, 2013).

As a good source of polyphenols with multiple biological activities, propolis has high potential to be used as an active agent that can be incorporated into films. Limited research on incorporation of propolis to enhance properties of packaging films and

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coatings has been published. Pastor, Sánchez-González, Cháfer, Chiralt, and González-Martínez (2010) investigated physical and antifungal properties of hydroxymethylcellulose based films containing propolis. Bodini, Sobral, Favaro-Trindade, and Carvalho (2013) studied properties of gelatin films added with propolis, and Torlak and Sert (2013) examined antibacterial effectiveness of chitosan-propolis coated polypropylene film. Nevertheless, research providing functional characterizations, including mechanical properties, oxygen and moisture barrier, structural property, antioxidant activity and antimicrobial property, of chitosan films incorporated with propolis extract (PE) has not been fully documented. Hence, this research aimed to develop and characterize chitosan films with enhanced functional properties for potential use as active food packaging by incorporating chitosan with propolis extract.

2. Materials and methods

2.1. Film preparation

Propolis was collected from Nan province in northern Thailand and extracted following the methods of Siripatrawan et al. (2013). Three grams of ground propolis were extracted using 100 ml of 30% ethanol aqueous solution. The solution was extracted at 50 °C in a water bath shaking incubator (New Brunswick Scientific, Edison, USA) at 200 oscillation/min for 24 h and then filtered through Whatman filter paper No. 1. The extract solution was concentrated using a rotary evaporator (Rotavapor R-200, BÜCHI Laboratory Equipment, Flawil, Switzerland) under reduced pressure at 45 °C.

Chitosan (Seafresh Industry Public Company Limited, Chumphon, Thailand) with 95% degree of deacetylation was used to prepare chitosan-based films. The chitosan-based film was prepared according to the procedure of Siripatrawan and Harte (2010) with slight modification. A film-forming solution was prepared by dissolving 2 g of chitosan powder into 100 ml of 1% acetic acid solution. Glycerol (Sigma Chemical Co., St. Louis, MO, USA) was added to the solution at 30% w/w of chitosan. The solution was heated at 60 °C in a water bath shaking incubator at 100 rpm for 30 min. The PE was dissolved in the film-forming solution to obtain concentrations of 0, 2.5, 5, 10 and 20% w/w of chitosan. The resulting solutions were homogenized using a homogenizer (D-79282, Ystral GmbH, Ballrechten-Dottingen, Germany). Air bubbles in the film-forming solutions were removed using a sonicator (Cole-Parmer, Vernon Hills, Illinois, USA). Each film-forming solution was cast on a ceramic plate. The obtained films were conditioned in a chamber at 25 °C and 50% relative humidity for 48 h prior to testing.

2.2. Color measurement

Color (L*, a*, b*) values were measured using a Minolta Chromameter (CR-300, Minolta Camera Co., Osaka, Japan). Films were cut into 15 × 2.5 cm and six readings at different positions on each film were measured. Five replications were conducted for each treatment and five film samples were used for each replication.

2.3. Water vapor transmission rate

Water vapor transmission rate (WVTR) of the films was determined following the ASTM standard test method (ASTM., 2003). Film samples, previously equilibrated at 50% RH for 48 h, were sealed in glass cups containing silica gel. The film-covered cups were placed in an environmental chamber set at 25 °C and 75% RH. The cups were weighed periodically until steady state was reached (±0.0001 g). The WVTR (g m^{-2} day^{-1}) of the films was determined using Eq (1). At least five replications of each film treatment were tested for the permeability coefficient (WVP). The WVTR of the films was then used to calculate the WVP (g mm m^{-2} d^{-1} Pa^{-1}) using Eq (2).

$$WVTR = \frac{\Delta w}{A\Delta t} \tag{1}$$

$$WVP = WVTR\frac{x}{\Delta p} \tag{2}$$

where Δw is the moisture weight gain, A is the tested film area, Δt is the definite time once steady state was reached, x is the film thickness and Δp is the partial water vapor pressure gradient between the inner and outer surface of the film in the chamber.

2.4. Oxygen transmission rate

A single film specimen was cut out to have a measurement area of 50 cm^2. O_2 transmission rate (OTR) was analyzed using MOCON OX-TRAN 2/20 devices (MOCON, Inc., USA) following the ASTM standard method (ASTM., 2003). The specimens were mounted onto the diffusion cells and a mixture of 98% N_2 and 2% H_2 was used as a carrier gas. OTR was used to measure the amount of oxygen passing through films when exposed to a gradient with partial O_2 pressure across the films. As the oxygen permeated through the film sample, it was picked up by the carrier gas and carried through a coulometric sensor. The amount of oxygen contained in the carrier gas at equilibrium was measured. The test conditions used in the OX-TRAN 2/20 system were 25 °C and 50% RH. Duplicate film samples were tested. OTR (Eq (3)) is expressed as the quantity (q) of O_2 molecules passing through a film surface area (A) during time (Δt) at steady state. Oxygen permeability coefficient (OP) of the films was then calculated using Eq (4):

$$OTR = \frac{q}{A\Delta t} \tag{3}$$

$$OP = OTR\frac{x}{\Delta p} \tag{4}$$

2.5. Mechanical properties

Tensile strength (TS) and percentage elongation at break (%E) were measured with an Instron Universal Testing Machine (Model 5655, Instron Corporation, Canton, MA, USA) following the ASTM Standard Test Method D 882-91 (ASTM., 2003). Each film strip (15 × 2.5 cm) was mounted between the grips of the Instron and tested with an initial grip separation of 5 cm and crosshead speed of 1 mm/s. TS (Eq. (5)) was calculated by dividing the maximum load (F_{max}) by the initial cross-sectional area (φ) of the film sample expressed as MPa. %E (Eq. (6)) was calculated as the ratio of the film extension (Δl) at the point of sample rupture to the initial length (l_0) of a sample and expressed as a percentage. Measurements represent an average of at least nine replications.

$$TS = \frac{F_{max}}{\Phi} \tag{5}$$

$$\%E = \frac{\Delta l}{l_0}100 \tag{6}$$