



Preparation and characterization of active films based on chitosan incorporated tea polyphenols

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ABSTRACT

Active films based on chitosan incorporated tea polyphenols with different concentrations were developed. Attenuated total reflectance Fourier transform infrared spectrometry was used to investigate the potential interactions between chitosan and tea polyphenols in the films. Physical properties of the chitosan films incorporated tea polyphenols, including density, moisture content, opacity, color, water solubility and water vapor permeability, were measured. Antioxidant activity of the films was characterized in terms of total phenolic content and 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging activity. The results indicated that the incorporation of tea polyphenols caused interactions between chitosan and tea polyphenols and gave rise to the films darker appearance. After the addition of tea polyphenols, the films showed increased water solubility and decreased water vapor permeability. Meanwhile, the incorporation of tea polyphenols enhanced the total phenolic content and 2, 2-diphenyl-1-picrylhydrazyl free radical scavenging activity of the films, i.e., increased the antioxidant activity of the films. But the antioxidant activity of the chitosan films incorporated tea polyphenols declined with time.

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1. Introduction

There has been a growing interest in recent years to develop active packaging to improve food safety and shelf life. Principal active food packaging involve oxygen scavenging, moisture absorption, carbon dioxide or ethanol generation, and antimicrobial systems (Coma, 2008). One of the commonly used methods to develop active food packaging is to incorporate active compounds and ingredients, such as antioxidants and antimicrobial agents, into packaging materials, providing the packaging system additional functions that do not exist originally. However, concerns on consumer health and environmental issues lead researchers to develop the active food packaging based on biodegradable materials and natural substances.

Chitosan, a natural carbohydrate copolymer [β -(1-4)-2-acetamido- α -glucose and β -(1-4)-2-amino- α -glucose units] yielded from deacetylation of chitin [poly- β -(1-4)-N-acetyl-2 α -glucosamine], is a biodegradable, non-toxic, and biocompatible polysaccharide. Due to its antimicrobial activity, chitosan has a great potential for applications in antimicrobial packaging (Aider,

2010; Dutta, Tripathi, Mehrotra, & Dutta, 2009). It has been proven that chitosan based films and coatings are effective in food preservation (Portes, Gardrat, Castellan, & Coma, 2009; Vázquez, Flores, Campos, Alvarado, & Gerschenson, 2009). Functional substances, such as rosemary essential oil and mint, have been incorporated into chitosan to enhance its antibacterial activity (Abdollahi, Rezaei, & Farzi, 2012; Kanatt, Chander, & Sharma, 2008a). Although chitosan is a promising biopolymer for active food packaging, it does not have significant antioxidant activity. Improvement of antioxidant activity of chitosan could expand its applications in active food packaging.

Tea polyphenols (TP) are extracted from tea and contain catechin, flavones, anthocyan, and phenolic acid, but catechin is the main component with more than 80% content (Huang, Huang, Liu, Luo, & Xu, 2007). Catechin mainly contains epigallocatechin-3-gallate (EGCG), epigallocatechin (EGC), epicatechin-3-gallate (ECG), and epicatechin (EC) (Yang, Lambert, & Sang, 2009). Research results demonstrated the antibacterial and antioxidant activities of TP, showing good prospects for their use as preservatives and antioxidants (He, Lv, & Yao, 2006). It has been showed that using TP as antioxidant or preservative could extend the shelf life of fresh mutton and fresh-cut lettuce (Kumudavally, Phanindrakumar, Tabassum, Radhakrishna, & Bawa, 2008; Martin-Diana, Rico, & Barry-Ryan, 2008). The antioxidant mechanism of polyphenols is principally attributed to their capacity in trapping reactive oxygen

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species and chelating metal ions, which could generate radicals through Fenton reaction (Osman, 2011).

Previous studies showed that antioxidant activity of chitosan, one of the main functions for active food packaging, could be improved through incorporating of natural substances, such as plant extracts (Ponce, Roura, Valle, & Moreira, 2008), green tea extract (Siripatrawan & Harte, 2010), rosemary essential oil (Abdollahi et al., 2012), *Zataria multiflora* Boiss essential oil and grape seed extract (Moradi et al., 2012). As a good source of polyphenols, TP could be used as an antioxidant and incorporated into chitosan films or coatings. Therefore, the aim of this study was to develop environmentally friendly active films from chitosan incorporated TP with different concentrations. Antioxidant activity in terms of total phenolic content and DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical scavenging activity and physical properties, including density, moisture content, opacity, color, water solubility, and water vapor permeability of the films were evaluated.

2. Material and methods

2.1. Materials

Chitosan with degree of deacetylation of 80–95% was supplied by Sinopharm Chemical Reagent Co. Ltd. (China). Tea polyphenols (TP) were obtained from Huzhou Rongkai Co. Ltd. (China). The TP used in this study was light yellow powder, purity $\geq 99\%$, caffeine $\leq 0.5\%$, and ash $\leq 0.5\%$. Catechins in the TP is $\geq 90\%$, and EGCG in the catechins is $\geq 70\%$. Glycerol, anhydrous CaCl_2 and KNO_3 were from Beijing Beihua Fine Chemicals Co. Ltd (Beijing, China). Gallic acid and Folin–Ciocalteu (F–C) reagent were from Shanghai Hualan Chemical Technology Co. Ltd. (China) and Shanghai Lida biotechnology Co. Ltd. (China), respectively. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was from Sigma–Aldrich Company (St. Louis, USA).

2.2. Preparation of films

The chitosan solution with concentration of 4 wt% was prepared by dissolving chitosan into 2% (V/V) acetic acid aqueous solution at 60 °C with stirring of 800 rpm. Glycerol, as a plasticizer, was added to the chitosan solution at concentration of 30 wt% of the chitosan. The TP solutions with different concentrations were prepared by mixing TP with distilled water at 60 °C and stirring with 200 rpm for 10 min. The film solutions were prepared by mixing the chitosan solution and the TP solutions at weight ratio of 1:1 to fabricate the chitosan films incorporated TP at concentration (weight percentage) of 10%, 20%, 30% and 40% of the chitosan. After stirred with 800 rpm for 60 min at 60 °C and degassed, desired amount of film solution was distributed into Petri dishes for casting and dried at 50 °C and 30% relative humidity for 24 h. The peeled films were kept in a chamber at room temperature and 75% RH for 48 h prior to experimental use.

2.3. Characterization

2.3.1. Attenuated total reflectance Fourier transform infrared (ATR-FTIR) analysis

ATR-FTIR spectra of the obtained chitosan/TP films were measured by using a Nexus 670 FTIR Spectrometer with an ATR attachment (Nicolet, USA) to investigate the interactions of chitosan and TP in the films. The measuring probe directly touched the surface of the films. A spectral resolution of 4 cm^{-1} was employed and 64 scans were acquired for each spectrum in the range of 4000 to 1000 cm^{-1} .

2.3.2. Density, moisture content and opacity

Film density was determined from the specimen weight and volume. The specimen volume was calculated from specimen area and thickness. The specimen thickness was measured by using a hand-held digital micrometer (Mitutoyo Absolute, Tester Sangyo Co. Ltd., Tokyo, Japan) with a precision of 0.001 mm at five different positions in each specimen and the average values were taken.

Moisture content of the films was determined by measuring weight loss of films upon drying in an oven at $105 \pm 1 \text{ }^\circ\text{C}$ for 24 h. All the tests were conducted in triplicate and the means were reported. The moisture content was calculated according to the following equation:

$$\text{Moisture content (\%)} = \frac{M_w - M_d}{M_w} \times 100\% \quad (1)$$

where M_w is the weight of the films conditioned in 75% RH to moisture equilibrium and M_d is dry weight of the films.

Opacity was determined according to the method of Park, Je, and Kim (2004) by measuring the film absorbance at 600 nm using a UV spectrophotometer (Ruili Analytical Instrument Company, Beijing, China). The films were cut into a rectangle piece and directly placed in a spectrophotometer test cell. An empty test cell was used as the reference. The opacity of the films was calculated by the following equation:

$$O = \frac{\text{Abs}_{600}}{d} \quad (2)$$

where O is the opacity, Abs_{600} is the value of absorbance at 600 nm and d is the film thickness (mm).

2.3.3. Color properties

The color of the films was evaluated by using a HunterLab ColorFlex (Xinlian Creation Electronic Co. Ltd., Shanghai, China). The color values of L^* (luminosity), a^* (negative-green; positive-red) and b^* (negative-blue; positive-yellow) were measured. A standard plate CX 2064 was used as standard. The total color difference (ΔE^*) and chroma (C^*) were calculated as follows:

$$\Delta E = \sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2} \quad (3)$$

$$C = \sqrt{a^2 + b^2} \quad (4)$$

where $\Delta L = L^*_{\text{standard}} - L^*_{\text{sample}}$, $\Delta a = a^*_{\text{standard}} - a^*_{\text{sample}}$, $\Delta b = b^*_{\text{standard}} - b^*_{\text{sample}}$. The color parameter values of the standard plate are $L^* = 94.52$, $a^* = -0.86$, and $b^* = 0.68$. Five measurements were taken on each film, one at the center and four around the perimeter.

2.3.4. Water solubility (WS)

The WS of the films was defined as the percentage of solved specimen dry matter after 24 h of immersion in distilled water and measured according to the method used by Khoshgozaran-Abras, Azizi, Hamidy, and Bagheripoor-Fallah (2012). The chitosan/TP films, previously equilibrated at 75% RH, were cut to $1 \text{ cm} \times 4 \text{ cm}$ strips, and immersed in 50 ml of distilled water and stirred at 100 rpm. After 24 h, the strips were taken out and dried at $105 \pm 1 \text{ }^\circ\text{C}$ for 24 h and the final dry weight (W_f) was measured. The initial dry weight (W_i) was determined by drying the strips in an oven at $105 \text{ }^\circ\text{C}$ to constant weight. All the tests were conducted in triplicate and the means were reported. The water solubility was calculated according to the following equation:

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