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# Physical and antioxidant properties of flexible soy protein isolate films by incorporating chestnut (*Castanea mollissima*) bur extracts



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#### ARTICLE INFO

Article history:
Received 13 November 2015
Received in revised form
14 March 2016
Accepted 15 March 2016
Available online 16 March 2016

Keywords: Polyphenol Film permeability Tensile strength Cross-linking Secondary structure

#### ABSTRACT

The physical and antioxidant properties of soy protein isolate (SPI) films were investigated incorporated with chestnut bur extract (CBE) at different levels, 20 g/kg, 50 g/kg, 80 g/kg, and 100 g/kg (based on SPI content). Increased protection against UV light and oxygen barriers were observed in the SPI films containing CBE. The antioxidant properties of SPI films were improved with the addition of CBE as determined by 2, 2-diphenylpicrylhydrazyl radical scavenging activities. The tensile strength of SPI/CBE film reached the maximum value of  $2.1\pm0.1$  MPa when 80 g/kg CBE was added. Fourier transform infrared spectra indicated that the addition of CBE influenced the SPI film mechanical properties by changing the distribution of secondary protein structures in the films. The cross-section of the SPI/CBE films viewed using scanning electron microscopy became more compact as the CBE level increased. These results suggest that SPI/CBE film is an ideal choice for food packaging and preservation.

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#### 1. Introduction

Food packaging films and containers made of petroleum-based plastics have increased the pressure on landfills from plastic solid wastes. Thus, consumers are expecting the development of new biodegradable and safe packaging materials from natural polymers to reduce environmental pollution (Koshy, Mary, Thomas, & Pothan, 2015). Such biopolymer may be protein, lipid, or polysaccharide, and the physical properties of the resulting films may be variable, depending on the type of biopolymer employed. Among the vegetable proteins, soy protein is the most important representative. Because of its low cost, availability, and complete biodegradability, SP could be developed into sustainable materials (Swain, Biswal, Nanda, & Nayak, 2004). The protein content of SPI is greater than those of other soy protein products, and SPI film possesses good barrier properties against oxygen and oils at low levels of relative humidity (RH), and has moderate mechanical properties, which permits it to be used in certain food coatings and packaging applications (Wang, Marcone, Barbut, & Lim, 2012b).

Natural tannins are comprised of a wide range of oligomeric and polymeric phenols, which are categorized as GRAS food additives commonly used to protect food nutrients against oxidative degradation. They are usually classified in hydrolyzable tannins and condensed tannins. Hydrolyzable tannins are composed of one molecule of sugar, generally glucose, joined to phenolic acid, while condensed tannins (i.e. proanthocyanidins) are polymers and oligomers comprising flavan-3-ol units. In China, the production of chestnut (*Castanea mollissima*) was  $\sim 9.25 \times 10^5$  metric tons in 2007, representing 3/4th of the total world production (Yao, Qi, & Wang, 2010). This generates a large amount of inedible waste material, namely shells and burs (spiny cover). The extraction of high-value components such as phenolic compounds from food waste can reduce the overall food processing costs (Baiano, 2014). However, because purified phenolic compounds are difficult to obtain and because extracts sometimes have better antioxidant activities than those of pure molecules, there is a growing interest in the use of plant extracts (Wang et al., 2012a). Chestnut bur (extracted with methanol/water 50:50 (mL: mL) at 75 °C) had been identified as hydrolyzable gallotannins as characterised by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (Fernández-Agulló, Freire, Antorrena, Pereira, & González-Álvarez, 2014). In addition to gallic acid esters of glucose, ellagic acid, and vescalagin/castalagin were found in chestnut bur extracts (Maria do Carmo et al., 2010). Barreira, Ferreira, Oliveira, and Pereira (2008) revealed that hot water extracts of chestnut skins had the highest antioxidant activity values, particularly for lipid peroxidation

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inhibition in the thiobarbituric acid-reactive substances, compared with chestnut flowers, leaf and fruit.

At present, few studies concerning the use of plant extracts containing vegetable tannins as antioxidants of SPI films for food packaging have been conducted. Although hot water is not the most efficient solvent for polyphenol, its use reduces both the cost and the usage of volatile organic solvents compared to conventional industrial production. Therefore, this work aimed to develop an antioxidant food packaging film by incorporating hot water extracts of chestnut bur into SPI film and to elucidate the impact of chestnut bur extract at different levels on physico-mechanical properties and antioxidant activity of SPI films.

#### 2. Materials and methods

#### 2.1. Materials

SPI powder (90 g protein, 5 g moisture, 4 g ash, 0.3 g crude fiber, 0.1 g fat/100 g SPI powder) was provided by Harbin High-Technology Soy Protein Co., Ltd, China. A saturated salt solution of potassium carbonate was used to control the 43% RH at room temperature. All the chemicals used in the experiments were of analytical grade and commercially available.

#### 2.2. Preparation of chestnut bur extracts

Chestnut burs were collected from a fruit-processing plant in Tianjin, China. The extraction condition of chestnut burs was as described by Barreira et al. (2008). Briefly, the burs were washed with distilled water and dried at 50 °C for 12 h. Then, the burs were smashed and passed through 0.18 mm—0.25 mm sieves. The burs (100 g for 2 L of water) were extracted at 100 °C for 30 min. Finally, the extract was recovered by vacuum filtration. The solvent was evaporated in a rotavapor, then the condensed solution was dried in a vacuum oven to obtain a dry powder for further experiments.

#### 2.3. Film formation

SPI films were prepared using a casting method modified slightly from Wan, Kim, and Lee (2005), SPI solution (60 g/L) was prepared by dissolving 6 g SPI powder in 100 mL distilled water. 500 g/kg glycerol (based on SPI content) was added into the solution, then CBE was added to the solution at levels of 0 g/kg (control film), 20 g/kg, 50 g/kg, 80 g/kg, and 100 g/kg (based on SPI content). Homogenization was performed using a high-shear mixer (AE300S-H, Angni Instruments, Shanghai, China) at 4000 rpm for 3 min. The pH was adjusted to 8 with 2 mol/L NaOH (YongDa Chemical Reagent, Tianjin, China), and film-forming solution were heated at 80 °C for 20 min, then cooled to room temperature. 80 mL of the film-forming solutions were poured into the plexiglass plates (230 mm  $\times$  230 mm  $\times$  30 mm). The solutions were dried for 12 h at 50 °C in a vacuum drying oven. The dried films were peeled off and conditioned again at 43% RH and 25 °C for 24 h prior to testing.

#### 2.4. Film characterization

#### 2.4.1. Color, light transmission and film transparency

The color of film was determined using a portable colorimeter (2600d, Xrite, Grand Rapids, USA). The CIELAB color space scale was used to obtain the color coordinates. Five measurements were taken on each film: one at the center and four around the perimeter. Light transmittance was measured at the ultraviolet and visible ranges (200–800 nm) using a UV–Vis spectrophotometer (UV-2600, Shimadzu, Kyoto, Japan). The tests were carried out in triplicate. The transparency value of the film was calculated using

the following equation:

Transparency value = 
$$-\log T_{600}/x$$
, (1)

where  $T_{600}$  is the fractional transmittance at 600 nm, and x is the film thickness (mm). According to the equation, a higher transparency value represents a lower transparency and a higher opacity.

#### 2.4.2. Moisture content and oxygen permeability

Moisture content (MC) was determined according to an oven drying method (Kanmani & Rhim, 2014). Each film sample was cut into square of 2 cm  $\times$  2 cm. Films samples were first weighed (W<sub>1</sub>), then placed into an oven at 105 °C for 24 h, and then reweighed (W<sub>2</sub>). Oxygen permeability (OP) of the film (50 cm<sup>2</sup>) was measured using Ox-Tran equipment (PERME OX2/230, Labthink, Jinan, China) according to the Chinese National Standard GB/T19789-2005. The tests were performed in continuous mode at 25 °C and 0% relative humidity. All tests were performed in triplicate.

The MCs of the films were calculated as follow:

$$MC(\%) = 100(W_1 - W_2)/W_1 \tag{2}$$

#### 2.4.3. Mechanical properties

Mechanical properties, including tensile strength (TS) and percentage of elongation at break (%E), were determined as described by Li, Miao, Wu, Chen, and Zhang (2014), using Auto Tensile Tester [XLW(PC), PARAM, Jinan, China], with a slight modification. Five samples (15  $\times$  150 mm) with initial grip lengths of 50 mm were used for testing, and the cross-head speed was set at 300 mm/min.

#### 2.4.4. Determination of antioxidant activity

Samples of 0.4 g of each film were placed in conical flasks containing 10 mL distilled water. These flasks were then continuously shaken at 100 rpm in orbital shakers set at three different temperatures, 15 °C, 25 °C, and 35 °C. Three sets of each film were tested for 10 h at each temperature. Then, the extract was filtered through a 0.45  $\mu m$  filter (Xinya Filter Membranes-organo System, Shanghai, China). The filtered liquid was transferred to a 50 mL flask and diluted with distilled water to a final volume of 50 mL. The antioxidant activities of the films at different temperatures were estimated by measuring the total phenolic (TP) contents and 2, 2-diphenylpicrylhydrazyl (DPPH) radical scavenging activities.

The TP contents of the films were determination by the Folin-Ciocalteu method of Genovese, Da Silva Pinto, De Souza Schmidt Goncalves, and Lajolo (2008) with a slight modification. Briefly, 1 mL of film solution was mixed with 0.4 mL of Folin-Ciocalteu reagent (Hero Chemical Company, Shanghai, China) and 4 mL distilled water. After 3 min at room temperature, 5 mL of 0.7 mol/L sodium carbonate solution was added, and the mixture was placed at room temperature for 2 h. Absorbance of the solution was measured at 765 nm. The TP acid content was expressed as gallic acid equivalents.

The antioxidant activities of the film samples were evaluated using DPPH free radical scavenging according to the procedure of Yamaguchi, Takamura, Matoba, and Terao (1998) with a slight modification. The film solution (1 mL) was added to 4 mL ethanol solution of DPPH (25 mg/L; Ruitaibio Company, Beijing, China) and mixed vigorously. After a 30 min incubation in the dark at room temperature, the absorbance was measured at 517 nm. The percent DPPH scavenging activity was calculated as follows:

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