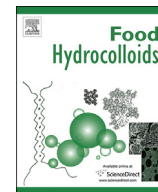




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# Preparation and characterization of antioxidant soy protein isolate films incorporating licorice residue extract

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## ABSTRACT

Antioxidant films were prepared by incorporating licorice residue extract (LRE) into soy protein isolate (SPI) films, and the effects of different concentrations of LRE (0, 10, 30, 50, and 70 g kg<sup>-1</sup> based on the weight of SPI) on the microstructure, physical properties, and antioxidant activities of SPI films were investigated. Fourier transform infrared spectroscopy indicated that the interaction between the SPI and LRE was via hydrogen bonds. The microstructure of SPI films with LRE was rough as observed by scanning electron microscopy. Moreover, the incorporation of LRE significantly decreased the monolayer moisture content, swelling degree, the water vapor and oxygen permeability of the SPI based films. The tensile strength of films was increased from 7.69 to 10.83 MPa with increasing content of LRE from 0 to 50 g kg<sup>-1</sup>. The films with LRE had excellent UV barrier properties, the surface color became deeper brown with increasing LRE content. In addition, the films with LRE released significantly more total phenolic content and antioxidant into alcoholic and fatty food simulants compared with control films, and possessed stronger 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) scavenging activities. SPI film incorporating LRE is an ideal active food packaging material for fatty food preservation.

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## 1. Introduction

There is currently increased interest in the application of renewable and biodegradable polymers in coating and packaging due to concern over the decline in petroleum-derived resources and the use of landfill sites for disposal (Leceta, Etxabide, Cabezudo, de la Caba, & Guerrero, 2014). Although traditional plastics packaging has excellent durability, and is an ideal material in the food industry, the waste plastics can result in serious environment problems due to its lack of biodegradability. In order to preserve fossil fuel resources and to lessen environmental pollution, safe and eco-friendly biopolymers should be considered in the development of food packaging materials, especially those from renewable agro-industrial by-products (Andrade, Ferreira, & Gonçalves, 2016).

Soy protein isolate (SPI), a by-product obtained from soybean oil processing, is prepared from defatted soybean flour. It contains more than 90% protein, attributed to its main constituents; 7S ( $\beta$ -

conglycinin) and 11S (glycinin) (Kumar, Sandeep, Alavi, Truong, & Gorga, 2010). SPI has been widely used in the food industry due to its nutritional value and low cost. Moreover, SPI owns good biodegradability, biocompatibility, and film-forming capacity. The SPI-based film has moderate mechanical properties and excellent oxygen and oil barrier properties that allow its application in food packaging and coating (Wang, Marcone, Barbut, & Lim, 2012).

Active packaging is an innovative concept that incorporates antioxidants, antimicrobial agents and/or nutrients to demonstrate not only barrier properties, but also some food preservation functions (Siripatrawan & Harte, 2010). Incorporation of antioxidants into packaging materials is important, since oxidation is one of the major problems affecting food quality (Siripatrawan & Noipha, 2012). Due to the health concerns of consumers, current research in antioxidant packaging is focused on the use of natural antioxidant compounds or the use of extracts rich in antioxidants, rather than using synthetic materials (Chang-Bravo, López-Córdoba, & Martino, 2014; Colín-Chávez, Vicente-Ramírez, Soto-Valdez, Peralta, & Auras, 2014). Recently, different natural antioxidants have been used to enhance the antioxidant properties of soy protein based films, such as tutin, epicatechin (Friesen, Chang, & Nickerson, 2015), red garpe extract

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(Ciannamea, Stefani, & Ruseckaite, 2016), and mango kernel extract (Maryam Adilah, Jamilah, & Nur Hanani, 2018).

Licorice is one of the oldest and most widely used herbal medicines in both Eastern and Western countries, having therapeutic effects on gastric ulcers, respiratory disorder, inflammation, liver disease, and adrenal fatigue (Lv et al., 2014; Zhang & Ye, 2009). It is also used as natural sweetening agent in the food industry (Isbrucker & Burdock, 2006). The principal active components in licorice are glycyrrhizin and its derivatives, which are isolated by aqueous extraction. The residue from this extraction is discarded as waste in most factories, which is a waste of resources as well as giving rise to environmental pollution. Moreover, this simple water treatment cannot sufficiently extract the flavonoids from licorice, so there are still significant amounts of flavonoids in the licorice residue. The pharmacological activities of flavonoids in licorice have drawn great attention, including anti-inflammatory, anti-tumor and antioxidant activities. Fu, Chen, Li, Zheng, and Li (2013) successfully isolated six flavonoids from extracts of licorice: 5-(1,1-dimethylallyl)-3,4,4'-trihydroxy-2-methoxychalcone, licochalcone B, licochalcone A, echinatin, glycycomarin and glyurallin B, and suggested that the licorice extract had useful antioxidant and anti-inflammatory properties. Tohma and Gulçin (2010) studied the total phenolic contents and radical scavenging activity of lyophilized ethanol extracts of licorice root and found that they had powerful and effective antioxidant activity. Thus the incorporation of ethanol extracted flavonoids from licorice residue as an antioxidant agent in food packaging is an interesting way to enhance the economic value of this waste. To the best of our knowledge, the combination of SPI and licorice residue extract (LRE) had never been studied.

Here we investigate the effects of incorporating LRE on the microstructure, moisture sorption isotherm, barrier (water vapor permeability, oxygen permeability), mechanical, optical, and swelling properties of SPI films. We also evaluate the release of total phenolic content and antioxidant activity (2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging assay) from the film into two different food simulants.

## 2. Materials and methods

### 2.1. Film-forming materials

SPI powder (90% protein) was supplied by Harbin High Technology Soy Protein Co. Ltd. (Harbin, China). Licorice roots were purchased from Yixiaotang Chinese medicine pharmacy (Bozhou, China). DPPH and ABTS were purchased from Sigma-Aldrich Chemical Co. Ltd. (St. Louis, MO, USA). Folin–Ciocalteu reagent was purchased from Hero Chemical Co. Ltd. (Shanghai, China).

### 2.2. Extraction of LRE

Licorice roots were extracted three times (2 h for each time) with boiling water. The licorice residues were dried in an oven at 60 °C. Then, the dried licorice residues (100 g) were powdered and extracted with 1 L of ethanol for 12 h in a conical flask with continuous shaking (100 rpm). The extracts were filtered through a filter cloth (500 mesh) and concentrated using a rotary evaporator at 45 °C. Finally, the LRE paste was dissolved in ethanol to give a solution in which the concentration of the LRE was 43.6 g/L.

### 2.3. Formation of SPI films

An SPI solution was prepared by dispersing SPI (6 g) and glycerol (3 g) in 90 mL of distilled water under magnetic stirring, and the pH was adjusted to 9.0 with 2.0 M NaOH. The resulting solution was

heated at 85 °C for 20 min. After that, LRE (0, 10, 30, 50, and 70 g per kilogram of SPI), was added to the cooled solution in 10 mL of ethanol, and stirred for 20 min. This would give films identified as LRE0, LRE10, LRE30, LRE50, and LRE70, respectively. The resulting solution was cast onto a Plexiglas plate (260 mm × 260 mm × 40 mm). The films were formed by drying at 55 °C for 12 h and conditioned at 43 ± 2% relative humidity (RH) for 24 h before testing.

### 2.4. Characterization

#### 2.4.1. Attenuated total reflection-fourier transform infrared (ATR-FTIR) spectroscopy

ATR-FTIR measurements were carried out by a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific Co., Ltd., MA, USA) from 4000 to 650 cm<sup>-1</sup> with a resolution of 4 cm<sup>-1</sup> and 32 scans.

#### 2.4.2. Scanning electron microscopy (SEM)

Scanning electron microscope (Philips-FEI, Netherlands, 10 kV) was used to observe the cross sections structures of films with gold coating. Samples were fractured in liquid nitrogen to expose the cross sections.

#### 2.4.3. Moisture sorption isotherms

The moisture sorption isotherms were determined by the gravimetric method of Xiao, Lim, and Tong (2012). Film samples were dehydrated in an air-circulating oven at 60 °C and then loaded in desiccators over different saturated salt solutions (LiCl, CH<sub>3</sub>COOK, MgCl<sub>2</sub>, Mg(NO<sub>3</sub>)<sub>2</sub>, NaCl, KCl, and BaCl<sub>2</sub>) which provided water activities (*a<sub>w</sub>*) in the range of 0.11–0.90. The change in mass was recorded until equilibrium was established. The results were adjusted to the Guggenheim–Anderson–De Boer (GAB) model using the Origin software (Version 8.0, Origin Lab Corporation, Northampton, USA):

$$X_{eq} = X_0 C K a_w / ((1 - K a_w)(1 - K a_w + C K a_w)) \quad (1)$$

where  $X_{eq}$  is the equilibrium moisture content (g H<sub>2</sub>O/g dry film),  $X_0$  is the moisture content in the monolayer (g H<sub>2</sub>O/g dry film),  $a_w$  is water activity, and  $C$  and  $K$  are related to the sorption heat of monolayers and multilayers, respectively.

#### 2.4.4. Water vapor permeability (WVP)

The WVP was performed according to the method of Shojaee-Aliabadi et al. (2014) with slight modification. The film samples were sealed to the cups (effective film area of 0.001662 m<sup>2</sup>) containing anhydrous calcium chloride [0% RH] and then the cups were conditioned at 25 °C and 75% RH. The cups were weighed periodically for 48 h. The WVP was calculated as follows:

$$WVTR = \Delta w / (A \Delta t) \quad (2)$$

$$WVP = WVTR \times x / \Delta p \quad (3)$$

where  $WVTR$  is water vapor transmission rate,  $WVP$  is water vapor permeability,  $\Delta w / \Delta t$  is mass change of the cup versus time (g s<sup>-1</sup>),  $A$  is effective film area (m<sup>2</sup>),  $x$  is film thickness (m), and  $\Delta p$  is partial water vapor pressure difference across the films (Pa).

#### 2.4.5. Oxygen permeability (OP)

The OP of the films was determined at 25 °C and 0% RH by oxygen permeability tester (Labthink, Perme OX2/230, Jinan, China). The film samples were placed on the sample chambers with an open testing area of 50 cm<sup>2</sup>. Oxygen (60 mL/min) flowed in one side of the sample and nitrogen (10 mL/min) flowed in the other side.

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