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Soy protein-based films incorporated with cellulose nanocrystals and pine needle extract for active packaging

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ABSTRACT

Soy protein-based films have some interesting properties of being biodegradable, biocompatible, and inexpensive. However, their weak mechanical property and high sensitivity to moisture are major hindrances to using this protein-based film for food packaging applications. This study aimed to develop soy protein-based films incorporated with cellulose nanocrystals (CNCs) and *Cedrus deodara* pine needle extract (PNE). The physical property, barrier capacity, and antioxidant ability of the films were evaluated and the films were further characterized using a release test, Fourier transform infrared spectroscopy, Raman spectroscopy, and scanning electron microscopy. The results show that the addition of CNCs lowered the moisture content of the film samples by disrupting the hydrogen bonds between N—H groups of soy proteins and water molecules. The filling effect of CNCs caused a decrease in elongation at break and an increase in tensile strength. When a high content of PNE (5–10%) was incorporated in the films, the water vapor permeability was decreased due to the reduction of hydrophilic domains in the film matrix. Moreover, the PNE-added films contained phenolic compounds and displayed strong antioxidant activities. These results demonstrate that CNCs and PNE can significantly enhance the mechanical property, antioxidant ability, and water vapor barrier capacity of soy protein-based films that can be used as an active food packaging material.

1. Introduction

Protein-based films have gained increasing attention in recent years owning to their biodegradability, good gas barrier property, and oil resisting capacity (Insaward et al., 2015). Among various proteins, soy protein isolate (SPI) is a popular choice as a raw material for preparation of protein-based films because SPI is abundant, inexpensive, and has high protein content (> 90%) (Cao et al., 2007). SPI films have been used for food production, food packaging and other applications. There has been growing interest recently in enhancing the functions of soy protein-based packaging materials to improve their mechanical properties, moisture resistance, and other physical properties (Li et al., 2016; Liu et al., 2017). Thus, there remains a need to explore the use of novel materials to improve the performance and active functions of soy protein-based films.

Cellulose nanocrystals (CNCs) are a new type of industrial crops and products that can be used to enhance the functions of soy protein-based films. CNCs are needle-like and highly crystalline cellulose particles that have an average length ranging from tens of nanometers to a few micrometers (George and Sabapathi, 2015; Kallel et al., 2016). CNCs are renewable and biodegradable, and possess superior mechanical strength. They have been widely used as a filling agent in packaging materials (Ooi et al., 2016). CNCs can be added in biopolymer-based films to enhance their mechanical properties and water vapor barrier capacity. It has been reported that the barrier properties and stiffness of a biocomposite film were significantly improved with the addition of CNCs (Luzi et al., 2016). Moreover, incorporating CNCs in chitosan or alginate films can increase the tensile strength and decrease the water vapor permeability (Khan et al., 2012; Huq et al., 2012).

Botanical extract is a key component for improving active functions of biocomposite films. It has been reported that polysaccharide-based films filled with thyme or rosemary extracts displayed strong antioxidant effects (Talón et al., 2017; Piñeros-Hernandez et al., 2017). In addition, a biocomposite film containing milk thistle extract showed remarkable antioxidant capacity, good water resistant property, and water vapor barrier property (Ghelejlu et al., 2016). For example, *Cedrus deodara*, a traditional medical plant of the family Pinaceae, contains bioactive extracts that can be used in active packaging materials. *C. deodara* is widely grown in the Northeast China, South Korea, Japan, and to the west of the Himalayas (Kumar et al., 2011). Pine needles of

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C. deodara have been used in conventional Chinese tea products for hundreds of years. Recently, the bioactivities of *C. deodara* pine needles have received much attention. It has been reported that the *C. deodara* pine needle extract (PNE) displayed remarkable free radical scavenging capacity, inhibitory effect on peanut oil oxidation, antibrowning activity in a fresh-cut apple model, and antiseptic ability in tomato juices (Zeng et al., 2011; Zeng et al., 2012; Yu et al., 2014). However, the application of PNE in food packaging has not been investigated yet.

The objectives of this study were to develop soy protein-based films incorporated with CNCs and PNE and evaluate their enhanced active functions for applications in the industry. The mechanical properties, water vapor barrier capacity, and antioxidant ability of the films were evaluated by texture analysis, water vapor permeability estimation, and free radical-scavenging tests. To investigate the mechanism and effect of CNCs and PNE on the film matrix, the prepared film samples were further tested by a release test, Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, and scanning electron microscopy (SEM). To the best of our knowledge, this is the first study using CNCs, PNE, and soy proteins to prepare nanocomposites for food packaging applications.

2. Materials and methods

2.1. Materials

CNCs prepared from wood pulp (average length, 150 nm; average diameter, 7.5 nm) were obtained from CelluForce, Inc. (Montréal, Canada). SPI powders (protein content, > 90%) were provided by Bulksupplements.com (Henderson, NV, USA). *C. deodara* pine needles were collected from Chengdu, China and identified based on its morphological features. Gallic acid, 2,2′-azinobis-3-ethylbenzthiazoline-6-sulfonate (ABTS), Folin-Ciocalteu reagent, glycerol, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and anhydrous calcium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of pine needle extract

C. deodara PNE was prepared based on a previous method with some modifications (Yu et al., 2014). Briefly, the pine needles were washed, dried at 45 °C for 5 h, and turned into powder using a JYL-350 mixer (Jiuyang Co., Ltd., China). After 500 g of the powders were added in 70% (*v/v*) methanol solution, the mixture was stirred continuously for 24 h. The supernatant was collected, filtered and condensed under vacuum at 45 °C. The extraction processes were performed three times. The PNE was obtained with a yield of 6.12% (6.12 g PNE/100 g dried powders) after freeze-drying and stored at 4 °C for further use.

2.3. Total phenolic content of extract

The phenolic content in the PNE was measured by a reported method with some modifications (Jia et al., 1999). Briefly, PNE solution (0.1 mL) and sodium carbonate solution (2 mL, 20 mg/mL) were mixed well and reacted at 25 °C for 2 min. After the addition of Folin-Ciocalteu reagent (0.9 mL), the mixture was kept at 25 °C for 30 min. The absorbance at 750 nm was then determined. Gallic acid (GA) was used as the standard and the total phenolic content was expressed as "mg GA equivalent/g PNE".

2.4. Film preparation

SPI solution (6%, *w/v*) was prepared by dissolving in distilled water. Glycerol (60%, *w/w*, based on the dry weight of SPI) was then added and mixed well with the SPI solution. The pH of the mixture was modified to 10.0 using a 0.1 M sodium hydroxide solution. After the mixture was incubated at 85 °C for 30 min and cooled down at room temperature, CNCs (15%, *w/w*, based on the dry weight of SPI) were added and stirred for 20 min. Subsequently, PNE (2.5, 5, 10%, *w/w*, based on the dry weight of SPI) was incorporated in the solution and dispersed well by stirring for 20 min. The film-preparing solutions (30 mL) were casted to polystyrene square petri dishes (120 mm × 120 mm × 17 mm) followed by drying at 37 °C for 48 h. The dried films were peeled off and stored at 25 °C and 50% relative humidity for at least 2 d before further analysis. A total of 5 different types of films were made and displayed as follows: soy protein film without CNCs and PNE (S), soy protein film with CNCs (SC), soy protein film with CNCs and 2.5% of PNE (SCE1), soy protein film with CNCs and 5% of PNE (SCE2), and soy protein film with CNCs and 10% of PNE (SCE3).

2.5. Color measurement

The color of soy protein-based films was measured using a portable colorimeter (Model, CR-410; Konica Minolta Sensing, Inc., Japan). The calibration was performed through a standard white plate (L_1*, 97.79; a_1*, 0.02; and b_1*, 1.92). Each film was placed on a standard plate before its L*, a*, b* values were recorded. The total color difference was determined based on the following equation: $\triangle E = [(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2]^{1/2}$, where L_1*, a_1*, and b_1* are the color parameters obtained from the white plate and L_2*, a_2*, and b_2* are the color parameters measured from the film samples.

2.6. Moisture content

The film sample (100 mg) was placed in a previously dried weighing glass jar and then incubated at 105 °C until a constant weight was obtained. The moisture content was estimated using the following equation: Moisture content (%) = $(m_a - m_b)/m_a \times 100\%$, where m_a is the initial film weight (g) and m_b is the dry film weight (g).

2.7. Mechanical properties

The mechanical properties of film samples were analyzed via a tensile assay using a texture analyzer (Model, TA-XT2i; Texture Technologies Corp., UK). The films were cut into pieces with an identical size (20 mm × 70 mm) and fixed by grips. The initial distance between grips was 50 mm. The stretching speed of upper grip was set to be 1 mm/s until the rupture of film samples. The film thickness was measured using a digital electronic micrometer (Model 35-025;

Table 1
Thickness, color and moisture content of soy protein-based films.

Sample	Thickness (mm)	L^*	a^*	b^*	$\triangle E$	Moisture content (%)
S	0.16 ± 0.02^{a*}	91.61 ± 0.47^{a}	−2.03 ± 0.08^{b}	18.69 ± 0.93^{d}	17.99 ± 1.04^{d}	24.69 ± 2.61^{a}
SC	0.18 ± 0.01^{ab}	90.29 ± 0.32^{a}	−1.81 ± 0.06^{a}	19.49 ± 0.73^{d}	19.20 ± 0.79^{d}	19.04 ± 1.30^{b}
SCE1	0.18 ± 0.02^{b}	83.78 ± 0.72^{b}	−2.19 ± 0.04^{b}	26.22 ± 0.77^{c}	28.13 ± 1.03^{c}	17.60 ± 2.08^{b}
SCE2	0.18 ± 0.02^{b}	80.88 ± 1.36^{c}	−3.22 ± 0.24^{c}	31.77 ± 1.06^{b}	34.46 ± 1.55^{b}	16.79 ± 1.31^{b}
SCE3	0.18 ± 0.01^{b}	77.59 ± 0.96^{d}	−4.79 ± 0.07^{d}	38.62 ± 0.95^{a}	42.17 ± 1.27^{a}	17.00 ± 0.66^{b}

* Each value is expressed as mean ± SD (n = 3). The data with various lowercase letters in each vertical column are significantly different ($P < .05$).