Incorporation of phenolic compounds, rutin and epicatechin, into soy protein isolate films: Mechanical, barrier and cross-linking properties

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A B S T R A C T

Edible films prepared from soy protein isolate (SPI), with and without the phenolic compounds, rutin and epicatechin, as cross linking agents, were tested for their mechanical, optical and water vapour barrier properties. The addition of rutin significantly increased puncture strength (9.3 N) over SPI alone (6.4 N) whereas epicatechin had no effect. Tensile strengths of SPI films with rutin and epicatechin were similar (35.1 MPa and 22.1 MPa, respectively) and significantly stronger than films without added phenolics (9.3 MPa). SPI films without phenolics showed the greatest flexibility, as measured by tensile elongation. The addition of epicatechin was found to increase water vapour permeability significantly to 2.3 g mm/m^2 h kPa from 1.7 g mm/m^2 h kPa for SPI alone whereas rutin decreased water vapour permeability to 1.2 g mm/m^2 h kPa. Films without phenolics had lower opacity values than had those with phenolics. Findings indicate that rutin and epicatechin may be used as a natural means for improving specific properties of SPI films.

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1. Introduction

The development of bio-packaging, using proteins, polysaccharides and/or lipids as a replacement of synthetic petroleum-based materials, has generated tremendous interest by the food industry, as it would alleviate environmental impacts (Zhang & Mittal, 2010), while offering the food product protection against physical, chemical and microbiological effects (Debeaufort, Quezada-Gallo, & Voilley, 1998; Falguera, Quintero, Jiménez, Muñoz, & Ibarz, 2011; Janjarasskul & Krochta, 2010). Soy protein, like other proteins, is an attractive film-forming material due to its ability to form many protein–protein linkages, leading to the formation of mechanically strong film networks (Zhang & Mittal, 2010). However, soy proteins can also have a negative impact on film properties, since the protein network is largely hydrophilic in nature, becoming weak under moist conditions and having poor moisture barrier capabilities (Gonzalez, Strumia, & Igarzabal, 2011). In contrast, films made from lipid and wax material have much greater water control, but have weak oxygen barrier and mechanical properties, as well as decreasing transparency as most lipids are somewhat opaque (Falguera et al., 2011).

Soy protein is dominated by two major storage (globulin) proteins: beta-conglycinin (150 kDa molecular mass) and glycinin

(350–400 kDa molecular mass) (Zhang & Mittal, 2010). Soy protein films are typically formed by first dispersing the protein powder, along with other ingredients, in water or aqueous alcohol (ethanol), followed by evaporation, which concentrates the protein and promotes protein–protein interactions until a network or film matrix is formed (Janjarasskul & Krochta, 2010). Some level of protein denaturation is typically required through heating, or an acid/alkaline pre-treatment, to help expose buried hydrophobic moieties to the surface for protein–protein interactions (Wittaya, 2012). Film properties can also be adjusted by altering the protein concentration and the solvent conditions within the film-forming solution (e.g., pH and salts) to further facilitate protein–protein interactions, adding lipids/waxes, plasticisers, or through the use of fixatives. Plasticisers (e.g., glycerol) are small molecules with low volatility which help overcome brittleness in the film by decreasing the intermolecular forces along biopolymer chains, which improves flexibility, elongation and tear resistance, while reducing toughness of the film (Yang & Paulson, 2000).

Cross-linking agents can improve the mechanical and water barrier properties of protein films by fixing the film to reduce its solubility, ability to swell and gas/water vapour permeability. Recently, phenolic compounds have been used as fixatives in film preparation, e.g., tannins and gallic acid (Orliac, Rouilly, Silvestre, & Rigal, 2002), ferulic acid (Ou, Wang, Tang, Huang, & Jackson, 2005) and phenolic-rich extracts from oregano (Pruneda et al., 2008), grape seed (Sivarooban, Hettiarachchy, & Johnson, 2008),

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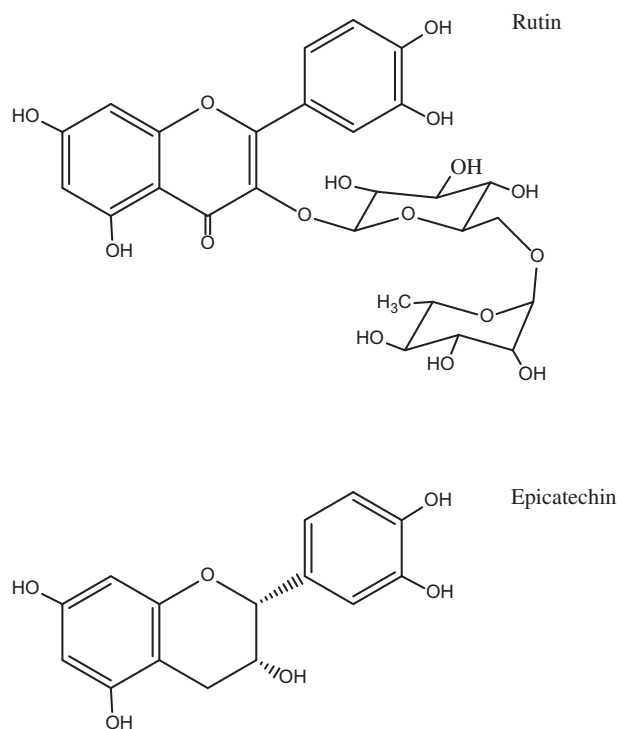 *E-mail address:* Michael.Nickerson@usask.ca (M. Nickerson).

Fig. 1. Structure of rutin and epicatechin.

blueberry (Zhang et al., 2010) and raspberry (Wang, Marcone, Barbut, & Lim, 2012). In the present research, rutin and epicatechin were chosen as 'model' phenolic compounds for naturally cross linking the SPI films (Fig. 1). Rutin is a flavonol glycoside that is made up of quercetin and the disaccharide rutinose (rhamnose and glucose). Rutin is naturally found in buckwheat but can also be found in fruits (Nguyen, Liu, Zhao, Thomas, & Hook, 2012). Epicatechin is a flavonol with an extra –OH group attached at the 5 and 7 positions when compared to rutin (Terao, 1999), and is not linked to a glucose molecule.

The overall goal of this research was to develop a SPI film cross-linked by rutin and epicatechin, to test the effect of the polyphenolics for improving the mechanical, optical and water barrier properties over SPI alone.

2. Materials and methods

2.1. Materials

Soy protein isolate (79.9% w.b. protein) used in this study was kindly donated by Cargill Health & Food Technologies, (Product Code ISP-221, Wayzata, MN, USA). All chemicals used in this study were reagent grade, and purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada). Milli-Q water was produced from a Millipore Milli-Q™ water purification system (Millipore Corporation, Milford, MA, USA). (+)-Rutin hydrate and (−)-epicatechin were purchased from Sigma–Aldrich Co. LLC. (St. Louis, MO, USA).

2.2. Film formation of SPI films

SPI films were produced using a casting method modified slightly from Chang and Nickerson (2014). In brief, a 5% (w/v) protein solution was prepared by dispersing the powder (corrected for protein content) in Milli-Q water and stirring for 10 min, using a mechanical stirrer at room temperature (21–23 °C), followed by a pH adjustment to 9.0 using 0.1 M NaOH. Glycerol was then added

(2.5%), followed by the addition of 0.05% (w/v) rutin or epicatechin, and an additional 10 min of stirring. The solution was heated to 85 °C for 30 min under constant stirring, using a water bath. Then the solution was strained, using cheesecloth to remove any remaining agglomerates and cast onto a polytetrafluoroethylene (PTFE) mould (10 cm length; 10 cm width; 1 mm depth). Any excess of the solution was removed from the mould, using a straight edge. The moulds were kept overnight in a gravity convection oven (APT.Line ED, Binder GmbH, Tuttlingen, Germany) at 50 °C. Films were then removed from the moulds and stored in desiccators at 54% humidity (using a saturated magnesium nitrate solution) at room temperature (21–23 °C) for 48 h. After conditioning, films underwent further testing. The final films were considered to be comprised of 5% SPI, 50% glycerol and 1% phenolics (or 50 mg phenolic per g protein). The concentrations of rutin and epicatechin used in this study were selected to be within the same range as in other works reported in the literature (Cao, Fu, & He, 2007; Fabra, Hambleton, Talens, Debeaufort, & Chiralt, 2011; Ou et al., 2005; Pruneda et al., 2008; Zhang et al., 2010). Films were made using triplicate solutions. After casting, the thickness of each film was measured, using a digital micrometre (Model 62379-531, Control Company, USA) with a precision of 0.01 mm. The mean was taken from ten different measurements across each film sample.

2.3. Mechanical testing

Puncture strength (PS, Newtons (N)) and deformation (PD, mm) of each film, with and without the addition of phenolics, were determined using a Texture Analyzer (Texture Technologies Corp., New York, USA), as described by Gontard, Guilbert, and Cuq (1992). Tests were done in triplicate, with 4 cm diameter discs cut from each pre-conditioned film. Films were placed on a puncture mould with a diameter of 65.6 mm and positioned under a smooth edged cylindrical probe (4 mm in diameter). The probe was moved perpendicularly onto the film surface at a cross-head speed of 1 mm/s until the film broke. The force–deformation data was recorded by a computer. PS was calculated as the maximum force (N) applied to the film before a puncture was achieved. PD was expressed as the change in length at the rupture point in the film.

Tensile strength (TS) and percentage tensile elongation (TE) at break of each film were obtained using the same instrument. TS can be defined as the maximum tensile strength which a material can sustain and is determined by the maximum load exerted on the test specimen during the procedure. TE was measured at the point where the film broke and was expressed as the percentage of change in the length of the original material between the grips of the testing machine (Chang & Nickerson, 2014). Films were tested in triplicate from 2.5 × 8 cm strips cut from 2 different pre-conditioned films. The film strips were mounted between the grips of the machine. The initial grip separation and cross-head speed were set to 50 mm and 300 nm/min, respectively. A computer was used to record the stress–strain curves.

2.4. Water vapour permeability

Water vapour permeability (WVP) was determined using the method described by Chang and Nickerson (2014). Each film was cut to fit on a WVP cup comprised of the following dimensions: outer cup height: 2.65 cm, outer cup radius: 2.50 cm, inner cup height: 2.00 cm and inner cup radius: 2.25 cm. These cups were then filled with 10 ml of saturated Mg(NO₃)₂ solution (54% relative humidity); the distance between the solution surface and the top of the cup was measured, and each cup containing the solution was weighed. The films were then placed on top of each cup, secured in place with rubber rings and screws, and placed in desiccators containing CaCl₂ to create 0% relative humidity. The