

Characterization of Cinnamaldehyde-Supplemented Soy Protein Isolate Films

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Abstract Soy protein isolate (SPI) films were supplemented with cinnamaldehyde (CA) at concentrations of 0.1-0.5 mL/5 g SPI. The effects of CA on film color, tensile strength (TS), percent elongation at break (E, %) and water vapor permeability (WVP) of SPI films were investigated. Generally, total color difference (ΔE), WVP, and TS of SPI films increased gradually, while E and TSM decreased significantly ($p < 0.05$) as the amount of cinnamaldehyde in the SPI films increased. Cinnamaldehyde can be used as a potential cross-linking agent for preparing SPI films by improving mechanical strength and water resistant properties.

Key words: soy protein isolate, cinnamaldehyde, cross-linking, protein films, mechanical properties

Introduction

Biopolymers such as polysaccharides, proteins, and lipids, either alone or in combinations, have potential as feedstock for degradable films. Proteins, as edible and degradable film-forming materials, have been studied. Protein films are effective lipid, oxygen, and carbon dioxide barriers. However, due to the inherent hydrophilicity of proteins and the substantial amounts of added plasticizers, protein films perform poorly as moisture barriers. Soy protein films have been modified using physical (1-4), chemical (5-7), or enzymatic treatments (8) to improve their moisture barrier property.

Aldehydes such as formaldehyde and glutaraldehyde promote inter- and intramolecular cross-linking in proteins (5, 6). Amino groups, sulfhydryl groups, the imidazole ring of histidine, and the phenolic ring of tyrosine have shown the ability to react with aldehydes (9). Soy protein is expected to be susceptible to aldehyde-induced cross-linking due to its high content of lysine. Formaldehyde treatment significantly increases tensile strength and puncture strength, and reduced the water solubility and water vapor permeability of soy protein films (5, 6). However, the inherent toxicity of formaldehyde restricts its use for improving the functional properties of films and coatings. Dialdehyde starch (molecular weight range of 300,000-5,000,000), which has lower toxicity than low molecular weight aldehydes (e.g., formaldehyde), improved the mechanical properties of soy protein isolate (SPI) films, but the problem of poor moisture barrier characteristics remained (7).

According to the literature (5-7), an aldehyde with a bulky molecular structure can be free from toxicity, but it is not effective as a cross-linking agent for protein films. Conversely, a low molecular weight aldehyde is an

effective protein cross-linker, but it is toxic. Cinnamaldehyde has been reported to function as an antifungal agent toward many filamentous fungi (10). However, its chemical properties, particularly its mode of action as a cross-linking aid, have not yet been investigated. The molecular weight of cinnamaldehyde (Mw:132.15) is higher than that of low molecular weight aldehydes (e.g., formaldehyde and glutaraldehyde), but much lower than the molecular weight of dialdehyde starch. Cinnamaldehyde is known to be non-toxic compound compared to other aldehydes such as formaldehyde and glutaraldehyde, and can be used as an effective cross-linking agent and edible additive (7).

The objective of this study was to prepare cinnamaldehyde-supplemented SPI films at various concentrations and to characterize the modified SPI films by measurement of total color differences (ΔE), tensile strength (TS), elongation at break (E, %), water vapor permeability (WVP) and total soluble matter (TSM). Such analysis is necessary to determine if cinnamaldehyde can be used as a non-toxic cross-linking agent for preparing SPI films with improved mechanical and water barrier properties.

Materials and Methods

Materials SUPRO[®] 1161, a soy protein isolate (SPI), was obtained from Protein Technologies International Company (St. Louis, MO, USA) and stored at 4°C prior to use. Glycerin (USP food grade) and sodium hydroxide were purchased from J.T. Baker Chemical (Phillipsburg, NJ, USA), and cinnamaldehyde (*trans*-, 99%) was purchased from Aldrich Chemical Company (Milwaukee, WI, USA).

Film preparation SPI films were prepared essentially according to the procedure described by Rhim and Weller (6) and Rhim and Lee (18). Film-forming solutions were prepared by dissolving 5 g of SPI in 100 mL of distilled water and 2.5 g of glycerin as a plasticizer. Sodium

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hydroxide (1 N) was used to adjust the pH of the film solution to 10.00 ± 0.01 . After the pH was adjusted, cinnamaldehyde was added directly to SPI film-forming solutions at levels of 0.1, 0.2, 0.3, 0.4, or 0.5 mL. The solutions were stirred for 1 hr and the pH was adjusted to 10.00 ± 0.01 . Each solution was heated for 15 min in a water bath at 75°C and strained through cheesecloth (Cheesecloth wipesTM; VWR[®], Chicago, IL, USA) to remove any bubbles and lumps. Film-forming solutions (90 mL) were cast on flat, level Teflon[®]-coated glass plates (21×35 cm). Films were peeled from the plates after drying at ambient temperature (25°C) for approximately 20 hr. Dried films were conditioned at 50% RH and 25°C for 24 hr.

Thickness Film thickness was measured to the nearest 2.54 m (0.1 mile) with a hand-held micrometer (B.C. Ames Co., Waltham, MA, USA). Five thickness measurements were taken on each water vapor permeability specimen, one at the center and four around the perimeter, and the mean was used in the WVP calculation. For TS calculations, five thickness measurements were taken along the length of each specimen and the mean was used.

Color Color values of the film were measured using a portable colorimeter (CR-300 Minolta Chroma Meter; Minolta Camera Co., Osaka, Japan). Film specimens were placed on a white plate and the HunterLab color scale was used to measure color: L = 0 to 100 (black and white), a = -80 to 100 (greenness and redness), and b = -80 to 70 (blueness and yellowness). Standard values for the white calibration plate were L = 96.89, a = -0.07, and b = 1.98. The change in color of the films was evaluated by comparing total color differences between films. Total color difference (ΔE) was calculated as:

$$\Delta E = [(L_{\text{standard}} - L_{\text{sample}})^2 + (a_{\text{standard}} - a_{\text{sample}})^2 + (b_{\text{standard}} - b_{\text{sample}})^2]^{0.5} \quad (1)$$

Tensile strength (TS) and percent elongation at break (E, %) TS and E (%) were determined using an Instron Universal Testing Machine (model 5566; Instron Corp., Canton, MA, USA) following the guidelines of ASTM Standard Method D 882-91 (11). Initial grip separation was set at 50 mm and cross-head speed was set at 500 mm/min. TS was expressed in MPa and calculated by dividing the maximum load (N) by the initial cross-sectional area (m^2) of the specimen. E (%) was calculated as the ratio of the final length at the point of sample rupture to the initial length of a specimen (50 mm), and was expressed as a percentage. TS and E (%) tests for each type of film were replicated five times.

Water vapor permeability Five film specimens were tested for each type of film. WVP ($\text{g}/\text{m}^2\cdot\text{hr}\cdot\text{Pa}$) was calculated as:

$$\text{WVP} = (\text{WVTR} \cdot l) / \Delta p \quad (2)$$

WVTR is the measured water vapor transmission rate ($\text{g}/\text{m}^2\cdot\text{hr}$) through a film specimen, l is the mean film specimen thickness (m), and Δp is the partial water vapor

pressure difference (Pa) between the two sides of the film specimen. WVTR was determined gravimetrically using a modification of ASTM Method E 96-95 (12). Film specimens were mounted on polymethylmethacrylate cups filled with 16 mL of distilled water up to 1.03 cm from the film underside. The design of the cups is described by Gennadios *et al.* (13). Cups were placed in an environmental chamber set at 25°C and 50% RH. A fan was operated in the chamber moving the air with a velocity of 196.3 m/min over the surface of films to remove the permeating water vapor. The weight of each cup was recorded six times at one-hour intervals. Linear regression was used to estimate the slope of this line in units of mass (g) per unit time (hr).

Total soluble matter TSM was calculated as the percentage of film soluble matter to initial dry matter during immersion in distilled water for 24 hr (7, 19). Film pieces (20×20 mm) were placed in 50 mL beakers containing 30 mL of distilled water. Beakers were covered with Parafilm 'M' wrap (American National CanTM, Chicago, IL, USA) and stored at 25°C for 24 hr. After discarding the water remaining in the beakers after 24 hr, residual film pieces were rinsed gently with distilled water. The pieces were then dried in an air-circulating oven (105°C) for 24 hr. The weight of solubilized matter was calculated by subtracting the weight of unsolubilized matter from the weight of initial dry matter and expressed as a percentage of the initial dry matter content using the following relationship:

$$\text{TSM} (\%) = [(S_0 - S) / S_0] \times 100 \quad (3)$$

S_0 = initial dry matter calculated by multiplying the initial sample weight by solid content and S = unsolubilized dry matter. The TSM measurement for each specimen was replicated three times.

Statistical analysis Experiments were conducted using a completely randomized design (CRD). ANOVA tables were generated for TS, E, WVP, and ΔE using the General Linear Model (GLM) procedure (SAS Institute, 1990), a package program of the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA). Significantly ($p < 0.05$) different means were separated with Duncan's multiple range test.

Results and Discussion

Color Soy protein films prepared without cinnamaldehyde (control) were smooth and transparent with a yellowish tint. Cinnamaldehyde-supplemented SPI films exhibited increasing total color differences. Such increases in total color difference values were greater with increasing amounts of cinnamaldehyde. Several researchers (6, 9) reported that aldehyde caused a darkening or browning in color of protein films due to interaction between proteins and aldehydes. Dialdehyde starch had a darkening or browning effect on SPI films (7). Lower molecular weight aldehydes, such as glutaraldehyde and glyceraldehydes, also produced a yellow coloration when reacted with various proteins. The ΔE values of cinnamaldehyde-

supplemented SPI films increased significantly ($p < 0.05$) up to 0.3 mL per 5 g of SPI and then reached a plateau (Fig. 1).

Tensile strength and elongation The TS of SPI films increased significantly ($p < 0.05$) due to the addition of cinnamaldehyde (Fig. 2). The mean TS of the control film was 3.45 MPa. TS increased up to 5.60 MPa due to the addition of 0.5 mL cinnamaldehyde. Generally, an inverse relationship existed between TS and E (%). The mean E (%) of the films decreased (Fig. 2) from 169.0 to 69.5% as the amount of added cinnamaldehyde increased from 0 to 0.5 mL, respectively. This indicates that cinnamaldehyde has a great cross-linking effect on the protein films since the TS and E values of cinnamaldehyde-supplemented protein films were more than two-fold higher and three-fold lower, respectively, compared to the control SPI film.

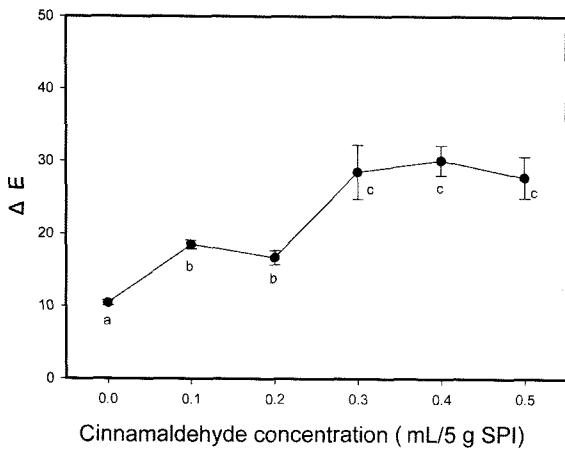


Fig. 1. Total color difference (ΔE) values of SPI films supplemented with cinnamaldehyde at concentrations of 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mL cinnamaldehyde/5 g SPI. Vertical bars represent the standard deviation and different letters show significant ($p < 0.05$) differences by Duncan's multiple range test.

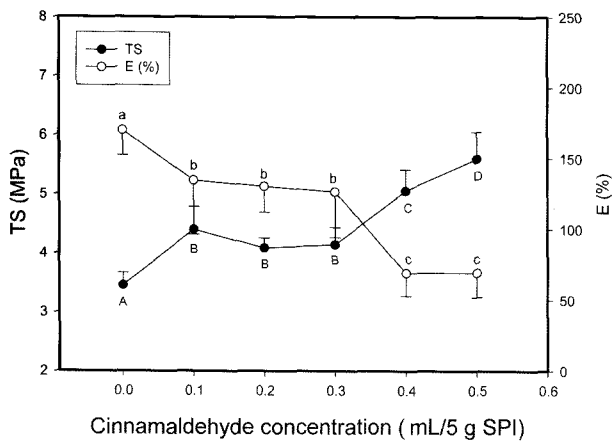


Fig. 2. Tensile strength (TS) and elongation at break (E) of SPI films supplemented with cinnamaldehyde at concentrations of 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mL cinnamaldehyde/5 g SPI. Vertical bars represent the standard deviation and different letters show significant ($p < 0.05$) differences by Duncan's multiple range test.

Rhim and Weller (6) and others (7) suggested that cross-linking between aldehyde groups and protein is the primary mechanism for increasing the mechanical strength of protein-based films.

Water vapor permeability The WVP of SPI films increased significantly ($p < 0.05$) with increasing amounts of cinnamaldehyde in the films (Fig. 3). The most significant increase in WVP was observed for SPI films supplemented with cinnamaldehyde at a concentration of 0.5 mL/5 g SPI. The thickness generally increased with the level of added cinnamaldehyde as the mass of material in the SPI film increased. There was a lot of deviation in the thickness values for SPI films with cinnamaldehyde added above the concentration of 0.4 mL/5 g SPI due to high viscosity of the film-forming solution. The solution became paste-like and was difficult to cast on a plate. In addition, a high standard deviation of thickness resulted in a high standard deviation of WVP for cinnamaldehyde-SPI films (Fig. 3). The WVP values of cinnamaldehyde-SPI films below 0.4 mL cinnamaldehyde/5 g SPI were not significantly different ($p > 0.05$) from the values of control films. The observed WVP values of SPI films across this range were fairly consistent near 15×10^{-6} g/m \cdot hr \cdot Pa, but relatively high compared to low density polyethylene (14), high density polyethylene (14), and cellophane (15) in terms of water resistance, which have typical WVP values of 0.329, 0.084, and 30.035×10^{-8} g/m \cdot hr \cdot Pa, respectively. The WVP values of SPI films increased at a concentration of 0.5 mL cinnamaldehyde/5 g SPI.

Ghorpade *et al.* (5) reported that formaldehyde-treated SPI films had slightly lower WVP values than untreated films, while Rhim *et al.* (7) reported dialdehyde starch (DAS)-SPI films had slightly greater WVP values than control SPI films. They used four different DAS concentrations of 5, 10, 16, and 20% (w/w). The WVP significantly increased to 6.05×10^{-6} g/m \cdot hr \cdot Pa, while SPI films without DAS averaged 5.40×10^{-6} g/m \cdot hr \cdot Pa. They suggested that this might have resulted from the bulky

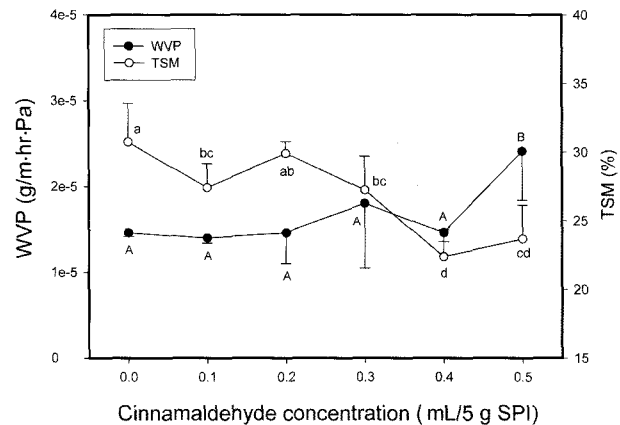


Fig. 3. Water vapor permeability (WVP) and total soluble matter (TSM) of SPI films supplemented with cinnamaldehyde at concentrations of 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mL cinnamaldehyde/5 g SPI. Vertical bars represent the standard deviation and different letters show significant ($p < 0.05$) differences by Duncan's multiple range test.

DAS molecules widening the interstitial spaces in the protein matrix, thus allowing for the increased diffusion rate of water molecules through the films. Cinnamaldehyde has a higher molecular weight (132.15) than formaldehyde (30.03) and also has a benzene ring in its structure. Although the molecular weight of cinnamaldehyde is much lower than that of DAS, its structural hindrance might have contributed to the increase in the diffusion rate of water molecules.

Total soluble matter The addition of cinnamaldehyde caused a substantial decrease in TSM of SPI films (Fig. 3). Specifically, the TSM decreased from 30.74% for control films to 23.67% for films supplemented with cinnamaldehyde at a concentration of 0.5 mL cinnamaldehyde /5 g SPI. In general, the decrease was greater with increasing amounts of cinnamaldehyde. Decreased solubility of protein due to cross-linking between proteins and cinnamaldehyde may be attributed to the reduced TSM of SPI films. Binding of cinnamaldehyde to hydrophilic sites such as guanidine, imidazole, and phenolic groups on protein chains, which are susceptible to cross-linking with aldehydes by associating with water through hydrogen bonding, caused the protein to lose solubility in water. The reduction in TSM of protein-based films caused by other aldehyde groups has been reported by several researchers. Marquié *et al.* (16) reported reductions in TSM of cottonseed protein films treated with formaldehyde, glutaraldehyde, or gossypol. In addition, the loss of solubility of hard and soft gelatin capsules due to formaldehyde-induced cross-linking has been reported by Digenis *et al.* (17). The reduction in TSM of SPI films by addition of cinnamaldehyde indicates an increase in water resistance of the SPI films. This characteristic of films is a desirable property for packaging materials in environments with high relative humidity or moisture.

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