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# Heat Curing of Soy Protein Films at Selected Temperatures and Pressures

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## Abstract

Vacuum and temperature effects on moisture content, water vapor permeability (WVP), color ( $L$ ,  $a$ ,  $b$ , and DE), tensile strength (TS), elongation (E), and total soluble matter (TSM) of soy protein isolate (SPI) films were examined. SPI films were cured at 60, 72.5, or 85 °C and at 101.3, 81.32, or 61.32 kPa for 24 h. As a result of heat-curing moisture content, WVP, E, and TSM decreased, and total color difference and TS increased. Pressure, individually and interactively with temperature, significantly affected film moisture content, TS, and TSM.

**Keywords:** soy protein, edible films, heat curing

## Introduction

An edible film is defined as a free-standing, thin layer of edible material formed separate from a food product and placed on or between food components. Krochta and Mulder-Johnston (1997) classified film functions as inhibiting migration of components in food, carrying food ingredients, improving mechanical integrity of food, or improving handling characteristics of food. To fulfill such functions, films need to have specific barrier and mechanical properties.

Films produced from renewable biological materials provide opportunities for innovative uses in food protection and preservation. A few products have already found commercial use; such as collagen for casings (Hood, 1987), waxes for fruits and vegetables (Kaplan, 1986), and zein-based coatings for nutmeats and candies (Alikonis, 1979). There have been studies on production and properties of films made from several polysaccharide, protein, and lipid substances (Krochta & Mulder-Johnston, 1997).

Proteins from several plant sources including soy, corn, wheat have been studied due to their film-forming capabilities. Soy protein films have poor moisture barrier properties due to the hydrophilic properties of soy proteins and the substantial amounts of hydrophilic plasticizer used in film preparation (Gennadios *et al.*, 1994). In spite of this, there is considerable interest

in edible and/or degradable soy films due to their environmentally friendly nature and their potential novel packaging uses in the food industry. Numerous studies have concentrated on improving mechanical and barrier properties of soy protein films through physical, chemical, or enzymatic treatments. Such studies included alkali treatments (Brandenburg *et al.*, 1993); sodium alginate or propylene glycol alginate alkylation (Shih, 1994; Rhim *et al.*, 1999); aldehydes cross-linking (Ghorpade, 1995; Rhein *et al.*, 2000); UV irradiation (Gennadios *et al.*, 1998; Rhim *et al.*, 1999); and enzymatic cross-linking (Motoki *et al.*, 1987; Stuchell & Krochta, 1994; Yildirim & Hettiarachchy, 1997).

Heat-treating protein films and coatings, or film-forming protein solutions, noticeably affected film properties. Cheftel and others (1985) reported thermal treatments of proteins at alkaline pH promoted formation of intra- and intermolecular cross-links. A variety of methods and substances have been employed to cure protein films by heating. The effects of heating on corn zein-epoxy resin (Howland, 1961; Howland & Reiners, 1962), gelatin and fibrin protein films (Julius, 1967), peanut concentrate films (Jangchud & Chinnan, 1999) and wheat gluten films (Kolster *et al.*, 1992) have been reported.

Gennadios and others (1996) showed that it was possible to improve the moisture barrier properties of soy protein films by heat-curing. Pérez-Gago and others

(1999) reported that heat-denatured whey protein films had higher tensile properties than native whey protein films. Studies showed that heat curing improved the mechanical toughness and moisture resistance of cast protein films made from corn zein (Julius, 1967), wheat gluten (Kolster *et al.*, 1992; Ali *et al.*, 1997), collagen (Weadock *et al.*, 1984), whey protein (Miller *et al.*, 1997), and soy protein (Gennadios *et al.*, 1996; Rangavajhyala *et al.*, 1997; Rhim *et al.*, 2000). These results suggest that covalent cross-linking, caused by heat denaturation of protein, is responsible for film water insolubility and higher tensile properties.

Based on the results summarized above, it was postulated that heat treatment under vacuum would influence the curing process and further enhance the resulting film properties. Reduced pressure during curing should increase the rate of film drying compared to curing methods that use heat at atmospheric pressure. Level of cross-linking may be influenced by drying rate. Therefore, our objective was to determine the effects of pressure and temperature, during a 24-h heat-curing process, on film color, tensile strength, elongation at break, water vapor permeability, moisture content, and total soluble matter of cast soy protein isolate films.

## Materials and Methods

### Film preparation

Film-forming solutions were prepared by mixing 100 mL of distilled water, 5 g of SPI (Supro 620, Protein Technologies International, St. Louis, MO, USA), and 2.5 g of glycerin (U.S.P. food grade, Mallinckrodt, Paris, KY, USA). Sodium hydroxide (1 mol/L) was used to adjust the pH to  $10.0 \pm 0.01$ . After pH was adjusted, solutions were held for 15 min in a water bath at 75 °C and strained through cheesecloth (Cheesecloth Wipest, VWR Scientific Products, Chicago, IL, USA) to remove any bubbles and lumps (only minuscule amounts were present). The film-forming solutions (90 mL) were cast on flat, level Teflon®-coated glass plates (21 cm×35 cm). Films were peeled from the plates after drying at ambient temperature for about 20 h. Dried films were conditioned at 50% RH and 25 °C for 24 h. The conditioned films were then cured in a vacuum chamber (National Appliance Co. Portland, OR, USA) at  $60 \pm 4$ ,  $72.5 \pm 4$ , or  $85 \pm 4$  °C under absolute pressures of 101.3 (atmospheric pressure), 81.32, or 61.32 kPa for 24 h. Pressures of 81.32 and 61.32 kPa were reached after about 10 and 20 min, respectively. After film curing, films were conditioned again at 50% RH and 25 °C for 48 h prior to testing.

### Moisture content

Pieces (100–200 mg) of cured film were prepared by cutting. Cured film pieces were immediately weighed and dried for 24 h in an air-circulating oven at 105 °C to determine moisture content (MC). Also, the MC values of cured films after being conditioned at 50% RH and 25 °C

for 48 h were determined by drying film pieces (200–300 mg) at 105 °C for 24 h. MC values were determined, in triplicate, for each type of film as a percentage of initial film weight lost during drying and were reported on a wet weight basis (Ghorpade *et al.*, 1995).

### Thickness

Film thickness was measured to the nearest 2.54 μm (0.1 mL) 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 water vapor permeability calculation. For tensile testing, five thickness measurements were taken along the length of each specimen and the mean was used in calculating film tensile strength.

### Color

Color values of films 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 = 96.86, a = -0.07, and b = 1.98. The change of color was evaluated by comparing total color differences between films. Total color difference ( $\Delta E$ ) was calculated as:

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

### Tensile strength and percentage elongation at break

Tensile strength (TS) and elongation at break ( $E$ ) were both measured with an Instron Universal Testing Machine (Model 5566, Instron Corp., Canton, MA, USA) following the guidelines of ASTM Standard Method D 882-91 (ASTM, 1995a). 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<sup>2</sup>) of the specimen.  $E$  was calculated as the ratio of the final length of the point of sample rupture to the initial length of a specimen (50 mm), as a percentage. TS and  $E$  tests for each type of film were replicated five times.

### Total soluble matter

TSM was expressed as the percentage of film dry matter dissolved during immersion in distilled water for 24 h. Measurement of TSM followed a modification of Rhim's method (Rhim *et al.*, 2000). Film pieces (20×20 mm<sup>2</sup>) were placed in 50-mL beakers containing 30 mL of distilled water. Beakers were covered with Parafilm 'M' wrap (American National Can™, Chicago, IL, USA) and stored at 25 °C for 24 h. After discarding the wa-

ter remaining in the beakers after 24 h, residual film pieces were rinsed gently with distilled water. Pieces were then dried in the air-circulating oven (105 °C) for 24 h. The weight of dissolved dry matter was calculated by subtracting the weight of insoluble solid matter from the initial weight of solid matter.

#### Water vapor permeability

Five film specimens were tested for each type of film. WVP (g m/m<sup>2</sup> h Pa) was calculated as

$$\text{WVP} = (\text{WVTR} \cdot L) / \Delta p$$

where WVTR was the measured water vapor transmission rate (g/m<sup>2</sup> h) through a film specimen, *L* was the mean film specimen thickness (*m*), and  $\Delta p$  was 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 (ASTM, 1995b) as described by Gennadios and others (Gennadios *et al.*, 1998). Film specimens were mounted on polymethylmethacrylate cups filled with 16 mL of distilled water up to 1.03 cm from the film underside. 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 velocity of 196.3 m/min over the surface of the films to remove the permeating water vapor. The weights of the cups were recorded six times at 1-h intervals. Linear regression was used to estimate the slope of this line in g/h.

#### Statistical analysis

The experimental design was a completely randomized design (CRD) of nine treatments (3 pressures  $\times$  3 temperatures). An ANOVA table was generated for each tensile strength, percent elongation, water vapor permeability, and total soluble matter using the general linear model (GLM) procedure, 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

#### Moisture content

Temperature and pressure, individually and interactively, affected MC of SPI films after heat curing ( $P < 0.05$ ). Mean values of film MC significantly ( $P < 0.05$ ) decreased from  $16.6 \pm 2.3$  to  $15.4 \pm 3.9$  to  $14.3 \pm 1.5$  (g/100 g) for pressures of 101.3, 81.32, and 61.32 kPa, respectively. For temperatures of 60, 72.5, and 85 °C, mean MC values significantly decreased from  $18.1 \pm 2.1$  to  $15.4 \pm 1.3$  to  $12.9 \pm 2.2$  (g/100 g), respectively. The mean MC (wet basis) of unheated SPI films from a parallel study (Kim *et al.*, 2001) after storage at 50% RH and 25 °C for 24 h was  $27.06 \pm 2.04$  (g/100 g). As heating temperature increased and pressure decreased, film MC values significantly decreased ( $P < 0.05$ ), as shown in **Table 1**. The

**Table 1.** Moisture content (g/100 g) mean values of soy protein isolate films heat-cured for 24 h at select temperatures and pressures

Pressure (kPa)	Temperature		
	60 °C	72.5 °C	85 °C
101.3	$18.80 \pm 1.17a$	$19.84 \pm 1.02a$	$14.60 \pm 2.90bc$
82.32	$15.70 \pm 0.69b$	$15.74 \pm 1.07b$	$14.74 \pm 0.98bc$
61.32	$15.09 \pm 1.72bc$	$10.79 \pm 1.86d$	$12.82 \pm 0.24cd$

*a-d*: Any two means followed by the same small-case letter are not significantly ( $P > 0.05$ ) different by Duncan's multiple range test.

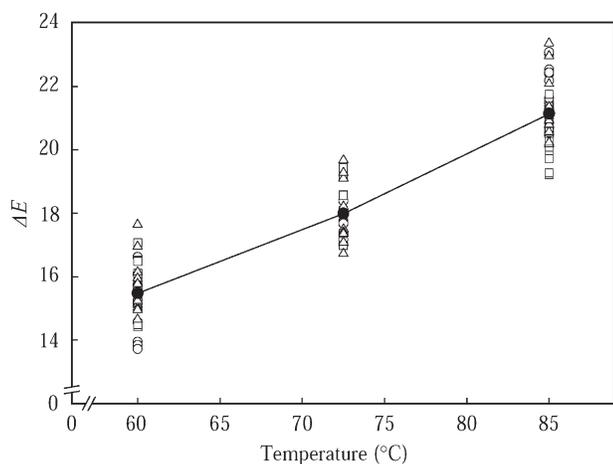
lowest MC ( $10.79 \pm 1.86$  g/100 g) was observed at the temperature and pressure combination of 72.5 °C and 61.32 kPa, respectively. Heat-cured films did not absorb water up to the original MC of unheated films. This suggested the development of cross-links between hydrophilic sites along SPI protein chains, thereby decreasing protein access to water molecules. Heat treatment induces crosslinking of protein molecules (Cheftel *et al.*, 1985) and the resulting structure restricts water uptake. Decreased water uptake was documented for propylene glycol alginate-treated SPI (Rhim *et al.*, 1999) and for SPI films heat cured at atmospheric pressure (Gennadios *et al.*, 1996).

#### Color

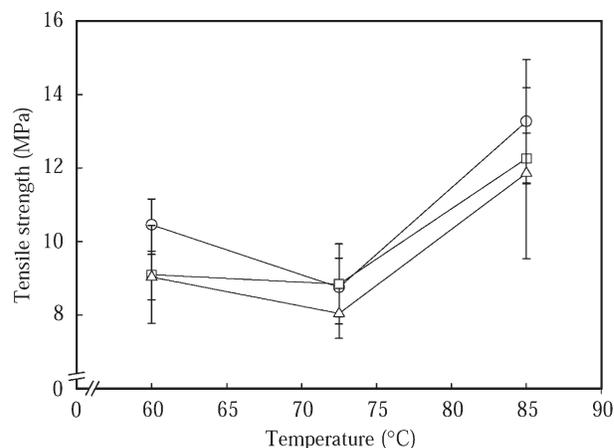
The total color difference ( $\Delta E$ ) of heat-cured films significantly increased with increasing curing temperature ( $P < 0.05$ ) as shown in **Figure 1**. Heated films became more yellowish (higher positive *b* values of  $14.90 \pm 0.80$ ,  $17.67 \pm 0.91$ , and  $21.00 \pm 1.07$ , respectively) as heating temperature increased. The *DE* of unheated SPI films of Kim and others (Kim *et al.*, 2001) was  $15.42 \pm 1.62$  and the mean *L*, *a*, and *b* color values were  $89.13 \pm 0.53$ ,  $-2.19 \pm 0.35$ , and  $15.15 \pm 1.67$ , respectively. Increased film yellowness also was reported for heat-cured wheat gluten and SPI films (Gennadios *et al.*, 1996; Rhim *et al.*, 1999, 2000), and for protein reacted with the cross-linking agent dialdehyde starch (Rhim *et al.*, 1998, 2000). Pressure and its interaction with temperature had no significant effects on  $\Delta E$  of SPI films ( $P > 0.05$ ).

#### Water vapor permeability

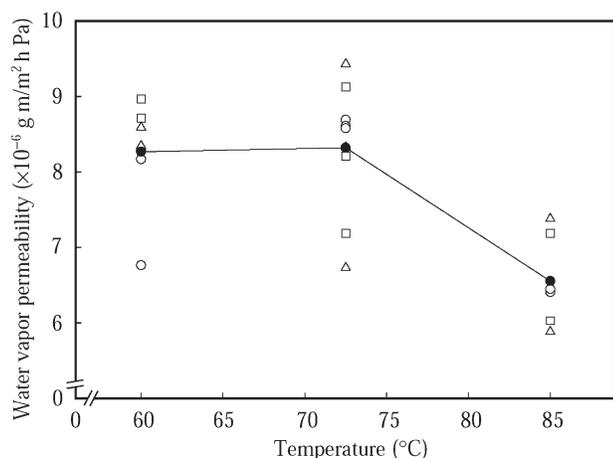
All heat-cured film samples had lower mean WVP values than the mean WVP of  $9.32 \times 10^{-6}$  g m/m<sup>2</sup>hPa ( $\pm 0.65 \times 10^{-6}$  g m/m<sup>2</sup> h Pa) for the unheated SPI films of Kim and others (2001). WVP of cured films was significantly ( $P < 0.05$ ) affected by temperature as shown in **Figure 2**. Thermal treatments of proteins apparently promoted formation of intra- and intermolecular cross-links of amino acid residues (Cheftel *et al.*, 1985). A significant ( $P < 0.05$ ) reduction in film WVP (decreased to  $6.55 \times 10^{-6}$  g m/m<sup>2</sup>hPa  $\pm 0.61 \times 10^{-6}$  g m/m<sup>2</sup> h Pa) was observed as heating temperature increased during film curing. The mean WVP value for films cured at 85 °C for 24 h was similar to the result of Gennadios and oth-



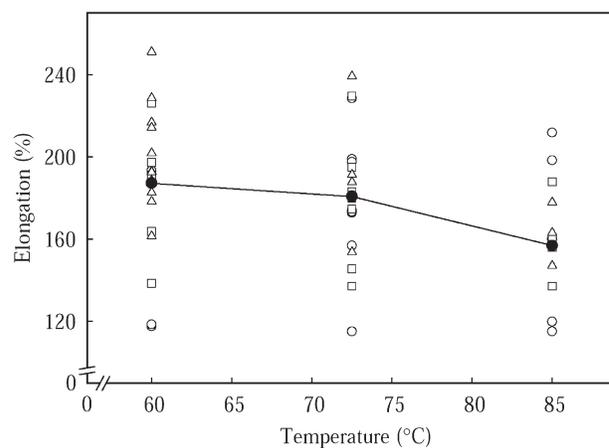
**Figure 1.** Total color differences of soy protein isolate films heat-cured at pressures (abs.) of 101.32 kPa (○), 81.32 kPa (□), or 61.32 kPa (Δ) for 24 h. The line through solid points (●) represents mean values of total color differences at each heating temperature.



**Figure 3.** Tensile strength (MPa) of soy protein isolate films heat-cured at pressures (abs.) of 101.32 kPa (○), 81.32 kPa (□), or 61.32 kPa (Δ) for 24 h.



**Figure 2.** Water vapor permeability values ( $10^{-6}$  gm/m<sup>2</sup> h Pa) of soy protein isolate films heat-cured at pressures (abs.) of 101.32 kPa (○), 81.32 kPa (□), or 61.32 kPa (Δ) for 24 h. The line through solid points (●) represents mean values of water vapor permeability at each heating temperature.



**Figure 4.** Elongation at break (%) of soy protein isolate films heat-cured at pressures (abs.) of 101.32 kPa (○), 81.32 kPa (□), or 61.32 kPa (Δ) for 24 h. The line through solid points (●) represents mean values of elongation (%) at each heating temperature.

ers (Gennadios *et al.*, 1996). They reported that the WVP of SPI film heat-cured for 24 h at 80 °C was  $6.2 \times 10^{-6}$  g m/m<sup>2</sup> h Pa. Reducing the pressure during heat-curing did not affect the WVP of SPI film ( $P > 0.05$ ). Gennadios and others (1996) suggested that the decrease in WVP of heated SPI films was attributable to formation of covalent links within the films and a decrease in protein hydrophilicity during heating.

#### Tensile strength and elongation

TS of heat-cured films increased significantly as curing temperature increased ( $P < 0.05$ ). Pressure affected TS ( $P = 0.04$ ) but there was no interactive effect of pressure and temperature ( $P > 0.05$ ). SPI films heat-cured at 85 °C had significantly higher tensile strength of 12.55 MPa ( $\pm 1.67$  MPa) than films heat-cured at 60 or 72.5 °C. Films heat-cured at 60 or 72.5 °C were not significantly different from each other ( $P > 0.05$ ). The mean

TS values for pressures of 101.3, 81.32, and 61.32 kPa were  $11.01 \pm 2.45$ ,  $9.98 \pm 1.86$ , and  $9.57 \pm 2.03$  MPa, respectively. Films cured at 81.32 or 61.32 kPa were significantly ( $P < 0.05$ ) different from those cured at 101.3 kPa, however, they were not significantly ( $P > 0.05$ ) different from each other (Figure 3). The TS of unheated SPI films was 5.96 MPa ( $\pm 1.10$  MPa) from a parallel study (Kim *et al.*, 2001). Heat treatment significantly ( $P < 0.05$ ) decreased film *E* (Figure 4). Temperature affected *E* of SPI films significantly ( $P < 0.05$ ). *E* values of SPI films were 187.2% ( $\pm 34.53\%$ ), 180.8% ( $\pm 29.31\%$ ), and 156.9% ( $\pm 24.89\%$ ) at temperatures of 60, 72.5, and 85 °C, respectively. Unheated SPI film had *E* of 214.3% ( $\pm 31.29\%$ ) (Kim *et al.*, 2001). Pressure did not affect *E* of SPI films during heat curing ( $P > 0.05$ ). Gennadios and others (1996) reported decreases in *E* as a result of heat treatment. They cured SPI films at 80 or 95 °C at atmospheric pressure. *E* values of films heated at 80 °C for 24 h were

not significantly ( $P > 0.05$ ) different from  $E$  values of films heated at 95 °C for 24 h. All observed  $E$  values were higher than those of about 30% reported by Gennadios and others (1996). Their mean thickness values ( $96.5 \pm 4.6 \mu\text{m}$ ) were greater than those ( $86.0 \pm 6.9 \mu\text{m}$ ) of this study. The reason may have been that our curing chamber was a closed system with no air circulation.

Similar to the results of our study, increases in film TS and decreases in film  $E$  also were observed for whey protein films heat-treated at 60, 70, or 80 °C (Miller *et al.*, 1997), wheat gluten films thermally-treated at 65, 80, or 95 °C (25), and SPI films heat treated at 80, 90, or 95 °C (Gennadios *et al.*, 1996; Rhim *et al.*, 2000). Miller and others (1997) reported that curing temperature and RH affected the rate of change of maximum TS,  $E$ , Young's modulus ( $Y_m$ ), and WVP of whey protein films. Specifically, they reported that curing time linearly affected TS and  $Y_m$  while decreasing  $E$  and WVP exponentially. The rate of exponential decay of  $E$  increased with increasing RH. They also suggested that the moisture content of a film determined its mechanical properties.

#### Total soluble matter

Films or packaging materials should maintain moisture levels within the packaged product. Moisture should not move to the film or packaging material and dissolve it. Therefore, TSM of a film is important for food packaging applications. TSM was calculated as the percentage of soluble matter to initial dry matter in each film sample.

Temperature and pressure significantly affected the TSM values of SPI films ( $P < 0.05$ ). There was no interactive effect of pressure and temperature ( $P > 0.05$ ). TSM decreased to the lowest value of 15.56 g/100 g ( $\pm 1.64$  g/100 g) at 85 °C and 61.32 kPa. This was the lowest value across all curing temperatures and pressures. Though these results were calculated as soluble solids, Rhim and others (2000) reported that SPI film heat-cured at 90 °C for 24 h had protein solubility of 0.03 mg/mL, which was only one-twelfth of the control (0.36 mg/mL). Heat-cured SPI films had lower TSM values than the value of 33.60 g/100 g ( $\pm 3.65$  g/100 g) for unheated SPI films (Table 2) from a parallel study (Kim *et al.*, 2001).

Reductions in TSM of protein films following heat treatments have been reported (Gennadios *et al.*, 1996; Rhim *et al.*, 1998, 2000). Rhim and others (2000) reported that lower moisture content could have contributed to reduced protein solubility of heat-cured films because water adsorption and film swelling probably took longer than in the case of unheated films. Mean TSM value of heat-cured films were 22.50 g/100 g ( $\pm 2.02$  g/100 g), 17.32 g/100 g ( $\pm 2.13$  g/100g), and 17.15 g/100g ( $\pm 2.04$  g/100 g) at curing temperatures of 60, 72.5, and 85 °C, respectively. Mean TSM values at a temperature of 60 °C were significantly ( $P < 0.05$ ) different from mean TSM values at temperatures of 72.5 or 85 °C. Mean TSM values of 20.65 g/100 g ( $\pm 3.64$  g/100 g), 18.85 g/100 g

**Table 2.** TSM (g/100 g) mean values of soy protein isolate films heat-cured for 24 h at select temperatures and pressures

Pressure (kPa)	Temperature		
	60 °C	72.5 °C	85 °C
101.3	24.62 $\pm$ 2.16a	18.95 $\pm$ 2.42cd	17.95 $\pm$ 1.40de
82.32	20.98 $\pm$ 0.80bc	17.21 $\pm$ 1.36de	17.95 $\pm$ 2.37de
61.32	22.09 $\pm$ 1.33ab	16.17 $\pm$ 2.00de	15.56 $\pm$ 1.64e

a-e: Any two means followed by the same small-case letter are not significantly ( $P > 0.05$ ) different by Duncan's multiple range test.

( $\pm 2.28$  g/100 g), and 18.54 g/ 100 g ( $\pm 3.53$  g/100 g) at pressures of 101.3, 81.32, and 61.32 kPa were not significantly different ( $P > 0.05$ ).

## Conclusions

Properties of SPI films were determined after 24 h of heat-curing at reduced pressure and increased temperature. Film MC, TS, and TSM were affected significantly by temperature and pressure, individually. MC was also significantly affected by the interaction of temperature and pressure. Temperature, but not pressure, did significantly affect  $\Delta E$ , WVP, and  $E$  of SPI films. Low pressure and high temperature resulted in SPI films with high TS and low TSM. Heat-curing at elevated temperatures with vacuum was able to modify the protein matrix of edible protein-based films such as to strengthen their structure. The structural changes in treated films apparently do not physically or chemically restrict water diffusion compared to untreated films.

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