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1996

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A. Gennadios

*University of Nebraska-Lincoln*, aris.gennadios@catalent.com

V. M. Ghorpade

*University of Nebraska-Lincoln*

Curtis L. Weller

*University of Nebraska-Lincoln*, cweller1@unl.edu

M. A. Hanna

*University of Nebraska-Lincoln*, mhanna1@unl.edu

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Gennadios, A.; Ghorpade, V. M.; Weller, Curtis L.; and Hanna, M. A., "Heat Curing of Soy Protein Films" (1996). *Biological Systems Engineering: Papers and Publications*. 94.  
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# HEAT CURING OF SOY PROTEIN FILMS

A. Gennadios, V. M. Ghorpade, C. L. Weller, M. A. Hanna

**ABSTRACT.** *Modification of soy protein film properties by heat-curing was studied. Glycerin-plasticized films were cast from alkaline aqueous solutions of soy protein isolate. Films were heated at 80 or 95°C for 2, 6, 14, or 24 h. Tensile strength (TS), elongation at break (E), moisture content (MC), water solubility (WS), water vapor permeability (WVP), and color of heated and control films were measured. Heated films had increased TS and +b (yellowness) Hunter color values and reduced E, MC, WS, and WVP values. These effects were enhanced as heating time and temperature increased.*  
**Keywords.** *Protein films, Soy protein, Edible films, Tensile strength, Water vapor permeability.*

**D**evelopment of biopolymer films and coatings from protein, polysaccharide, and lipid materials has received increased interest in recent years. In the midst of rising concerns over solid packaging waste, the renewable nature of biopolymer film ingredients renders such films particularly appealing for innovative uses in the field of packaging. Furthermore, as many of these film-forming biopolymers are edible, applications of edible packaging in the area of food packaging and preservation can be realized if edibility is maintained throughout the film production process. From an agricultural industry perspective, wide commercialization of biopolymer films would lead to further utilization of agricultural commodities employed as sources of film-forming materials.

Films and coatings based on proteins, such as wheat gluten, corn zein, soy protein, peanut protein, milk proteins, collagen, and gelatin, have been reviewed by Gennadios et al. (1994a). Several recent studies have concentrated on the development and property evaluation of soy protein isolate films in particular (Brandenburg et al., 1993; Gennadios et al., 1993a, 1994b; Shih, 1994; Stuchell and Krochta, 1994; Ghorpade et al., 1995). A variety of methods have been employed to modify soy protein film properties including treatment with alkali (Brandenburg et al., 1993); alkylation with sodium alginate (Shih, 1994); treatment with propyleneglycol alginate (Shih, 1994); enzymatic treatment with horseradish peroxidase (Stuchell and Krochta, 1994); acylation with

acetic and succinic anhydrides (Ghorpade et al., 1995); and treatment with formaldehyde (Ghorpade et al., 1995).

A few studies have shown that heat-treating prepared protein films and coatings or film-forming protein solutions had a noticeable effect on film properties. Heating of corn zein-epoxy resin films at elevated temperatures (i.e., 145°C for 30 min or 200°C for 5 min) increased film toughness and water resistance (Howland, 1961; Howland and Reiners, 1962). Increased water resistance also was reported by Julius (1967) for heat-cured zein, gelatin, and fibrin protein films. Kolster et al. (1992) heated wheat gluten films at 80°C and found that film stress increased, whereas film strain decreased, as the heating time increased. Roy et al. (1995) heated wheat gluten film-forming solutions at 55, 65, 75, 85, or 95°C for 10 min prior to casting. Reportedly, film tensile strength (TS) and percent elongation (E) at break increased, and solubility of film in water decreased with increasing temperature. In another study, Stuchell and Krochta (1994) cast films from unheated and heated (85°C) soy protein isolate film-forming solutions. Films from heated solutions had lower water vapor permeability and were more flexible than unheated control films.

The objective of this study was to determine the effect of heating on selected physical properties of soy protein isolate (SPI) films cast from alkaline aqueous solutions.

## MATERIALS AND METHODS

### FILM PREPARATION

Film-forming solutions were prepared by mixing 100 mL of distilled water, 5 g of SPI (ARPRO 1100, 93.5% d.b. protein content, obtained gratis from Archer Daniels Midland Company, Decatur, Ill.), and 3 g of glycerin (Fisher Scientific, Pittsburgh, Pa.). Glycerin was added as plasticizer to overcome film brittleness and to obtain free-standing films. Sodium hydroxide (1 N) was used to adjust the pH to  $11.0 \pm 0.1$ . Alkaline conditions have been shown to favor soy protein film formation, presumably, by aiding protein dispersion in the film-forming solutions (Gennadios et al., 1993a). However, high pH values may compromise the films' edibility. The solutions were held for 30 min in a 70°C constant

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Article was submitted for publication in July 1995; reviewed and approved for publication by the Food and Process Engineering Inst. of ASAE in October 1995. Presented as ASAE Paper No. 94-6552.

Journal Series No. 11206, Agricultural Research Division, Institute of Agriculture and Natural Resources, University of Nebraska, Lincoln.

The authors are **Aristippos Gennadios**, *ASAE Member*, Post-doctoral Research Associate, **Viswas Ghorpade**, *ASAE Member*, Assistant Professor, **Curtis L. Weller**, *ASAE Member Engineer*, Associate Professor, and **Milford A. Hanna**, *ASAE Member Engineer*, Professor, Dept. of Biological Systems Engineering, University of Nebraska, Lincoln. **Corresponding author:** Milford A. Hanna, Dept. of Biological Systems Engineering, University of Nebraska, 211 L.W. Chase Hall, East Campus, Lincoln, NE 68583-0726; telephone: 402-472-1634; e-mail: <bsen024@unlvm.unl.edu>.

temperature waterbath, strained through cheese cloth (Grade 40, Fisher Scientific, Pittsburgh, Pa.) to remove air bubbles formed during stirring, and cast on level Teflon®-coated glass plates. Films were peeled from the plates after drying at ambient temperature for about 30 h. Dried films had a thickness of  $96.5 \pm 4.6 \mu\text{m}$  (mean of 30 thickness measurements).

#### HEATING

Dried films were mounted on glass plates by applying masking tape around the film edges and heated at 80 or 95°C in an air-circulating oven for 2, 6, 14, or 24 h. Masking tape held films flat and prevented curling and rippling during heating. Property evaluation on curled film samples was meaningless since no reliable thickness measurements could be obtained. However, it should be noted that the practice of applying masking tape may have caused some stress development within the films. Following the heat treatment, films were appropriately conditioned for property evaluation. More severe heat treatments were not applied to the films to avoid evaporative losses of glycerin. The boiling point of pure glycerin at atmospheric pressure is 290°C, but drops substantially in the presence of water (Gregory, 1991).

#### MOISTURE CONTENT

Films were stored for three days in an environmental chamber maintained at 25°C and 50% RH (relative humidity). These conditions were similar to those of conditioning film specimens prior to tensile testing. Subsequently, samples of 300 to 400 mg were weighed into aluminum dishes and dried for 24 h in an air-circulating oven at 105°C. Moisture content (MC) was determined in triplicate for each type of film as percentage of initial film weight lost during drying and reported on a wet basis.

#### TENSILE STRENGTH AND ELONGATION

Tensile strength (TS) and elongation at break (E) were both measured with a model 5566 Instron Universal Testing Machine (Instron Corp., Canton, Mass.) following the guidelines of ASTM Standard Method D 882-88 (American Society for Testing and Materials, 1989). Film specimens (2.54 cm wide and 15 cm long) were cut. Five thickness measurements were taken along each specimen with a micrometer (B.C. Ames Co., Waltham, Mass.) and the mean of the five measurements was used in the TS calculation. Initial grip separation and cross-head speed were set at 10 and 5 cm/min, respectively. In an apparent deviation from ASTM Standard Method D 882-88, tensile testing of film samples was conducted, as quickly as possible, in ambient conditions rather than in the recommended Standard Laboratory Atmosphere of  $23 \pm 2^\circ\text{C}$  and  $50 \pm 5\%$  RH. Tensile strength was calculated by dividing peak load by initial specimen cross-sectional area. Elongation was expressed as the percentage of change of the original length of the specimen between the grips (10 cm). Prior to tensile testing, film specimens were conditioned for three days in an environmental chamber set at 50% RH and 25°C. Both TS and E values for each type of film were determined in triplicate with individually prepared, cast, and heated films as the replicated experimental units. Each TS and E replicate was the mean

of six tested sampling units (specimens) taken from the same film.

#### WATER SOLUBILITY

Films were stored for one week in a desiccator cabinet equilibrated to 0% RH with calcium sulfate (Drierite, Fisher Scientific, Pittsburgh, Pa.). Samples weighing 10 to 20 mg were immersed in 5 mL of distilled water in test tubes. Tube openings were covered with Saran® wrap and the samples were incubated at room temperature for 24 h with periodical gentle agitation. Protein concentration (mg/mL) was calculated using a bicinchoninic acid (BCA) protein assay (Smith et al., 1985). Water solubility (WS) results were expressed as the percentage of total protein detected in the solution (based on initial dry film weight after accounting for both the protein:glycerin ratio in the film and the protein content of the used commercial SPI product). Water solubility values were obtained in triplicate for each type of film.

#### WATER VAPOR PERMEABILITY

Film specimens  $7 \times 7$  cm were cut. Five thickness measurements were taken on each specimen, one at the center and four around the perimeter, and their mean was used as the specimen thickness in water vapor permeability (WVP) calculations. Prior to testing, all film specimens were conditioned for two days in an environmental chamber set at 25°C and 50% RH. Three film specimens, each from an individually cast and heated film, were tested for each type of film. The WVP ( $\text{g}\cdot\text{m}/\text{m}^2\cdot\text{s}\cdot\text{Pa}$ ) was calculated as:

$$\text{WVP} = (\text{WVTR}\cdot\text{L})/\Delta p \quad (1)$$

where

WVTR = measured water vapor transmission rate ( $\text{g}/\text{m}^2\cdot\text{s}$ ) through a film specimen

L = mean film specimen thickness (m)

$\Delta p$  = partial water vapor pressure difference (Pa) between the two sides of the film specimen

Water vapor transmission rate was determined gravimetrically using a modification of ASTM Standard Method E 96-80 (American Society for Testing and Materials, 1989). Film specimens were mounted on poly(methylmethacrylate) cups filled with distilled water up to 1 cm from the film underside. The design of the cups was described by Gennadios et al. (1994c). The cups were placed in an environmental chamber set at 25°C and 50% RH. A fan was operated within the chamber creating an air velocity of 198 m/min over the surface of the cups to remove the permeating water vapor. The weights of the cups were recorded six times at 1-h intervals. Steady state was reached within 2 h. Linear regression-derived slopes of the steady state (linear) portion of weight loss versus time curves were used to estimate WVTR. Because of the low water vapor resistance of protein-based films, the actual RH values at the film undersides during testing were lower than the theoretical value of 100%. These actual RH values at the film undersides inside the cups and the film WVP values were calculated after accounting for the resistance of the stagnant air layer between the film undersides and the water surface in the cups (McHugh et al., 1993;

Gennadios et al., 1994c). The mean of the initial and final stagnant air gap height was used in the calculations.

### COLOR VALUES

Color values of soy protein films were measured with a CR-300 Minolta Chroma Meter (Minolta Camera Co., Ltd., Osaka, Japan). This instrument is a tristimulus color analyzer with an 8-mm-diameter measuring area. Film specimens were placed on the surface of a white standard plate (Calibration Plate CR-A43) and Hunter L, a, and b color values were measured. The three color coordinates range from L = 0 (black) to L = 100 (white), -a (greenness) to +a (redness), and -b (blueness) to +b (yellowness) (Francis and Clydesdale, 1975). Total color difference ( $\Delta E$ ) was calculated from:

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

Standard values refer to the white calibration plate (L = 96.86, a = -0.02, b = 1.99). Measurements were taken in triplicate for each type of film.

### STATISTICAL ANALYSIS

Statistics on a completely randomized design were determined using the general linear models procedure in SAS (SAS Institute, 1988). Duncan's multiple range test ( $P < 0.05$ ) was used to detect differences among WVP and Hunter L, a, b, and  $\Delta E$  mean values at different combinations of heating time and temperature. Linear regression equations were fitted to E and WS data of heated films with the REG procedure in SAS (SAS Institute, 1988). Power regression equations were fitted to MC and TS data of heated films with the NLIN procedure in SAS (SAS Institute, 1988).

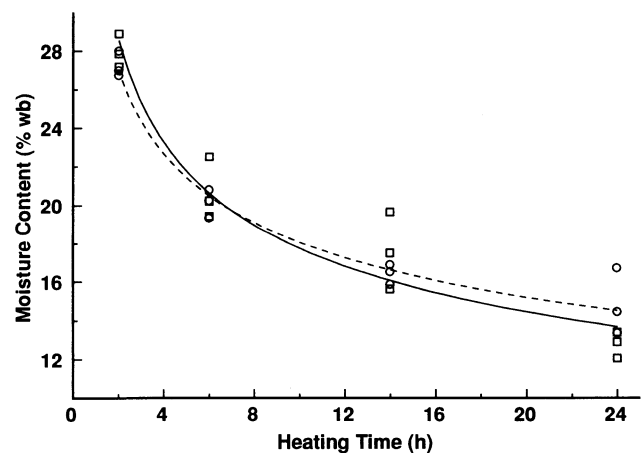


Figure 1—Moisture content values of soy protein isolate films heated at 80°C (○ with dashed line) and 95°C (□ with solid line) after storage at 50% RH and 25°C for three days. Power regression lines ( $r = 0.983$  and  $r = 0.966$  for 80 and 95°C, respectively) were fitted to the data.

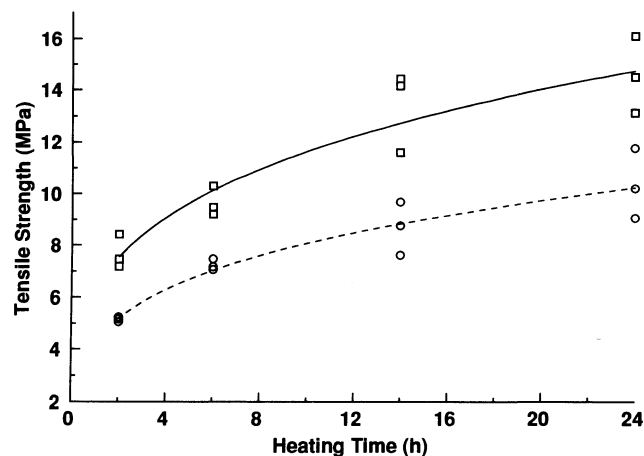


Figure 2—Tensile strength of soy protein isolate films heated at 80°C (○ with dashed line) and 95°C (□ with solid line). Power regression lines ( $r = 0.936$  and  $r = 0.936$  for 80 and 95°C, respectively) were fitted to the data.

## RESULTS AND DISCUSSION

### MOISTURE CONTENT

The MC (wet basis) of unheated SPI films after storage at 50% RH and 25°C for three days was  $28.5 \pm 0.8\%$ . As shown in figure 1, film moisture content decreased as a result of heating. Across all levels of heating time, there were no significant ( $P > 0.05$ ) differences in MC between films heated at 80°C and films heated at 95°C.

### TENSILE STRENGTH AND ELONGATION

Unheated SPI films had a TS (maximum tensile stress a film can sustain) value of  $3.25 \pm 0.06$  MPa. Film TS increased with heating time at both 80 and 95°C (fig. 2). Heat treatment at 95°C yielded films with significantly ( $P < 0.05$ ) higher TS than heat treatment at 80°C across all levels of heating time. On average, films heated at 95°C had TS values  $3.48 \pm 0.45$  MPa greater than films heated for the same time at 80°C.

In contrast to TS, heating decreased film E (a measure of a film's ability to stretch) from an initial value of  $111.9 \pm 12.4\%$  for unheated films (fig. 3). This heat-induced

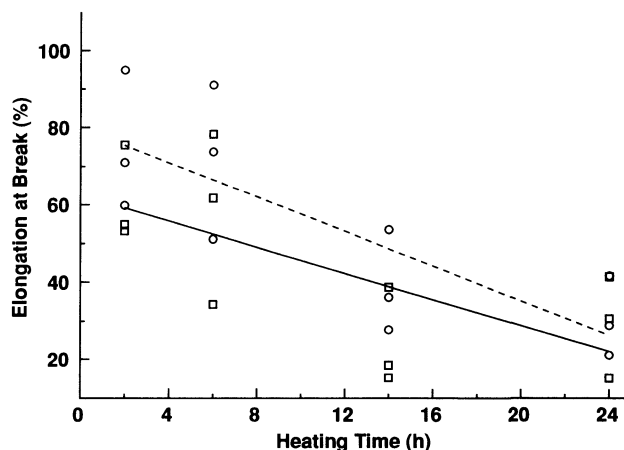


Figure 3—Elongation at break of soy protein isolate films heated at 80°C (○ with dashed line) and 95°C (□ with solid line). Linear regression lines ( $R^2 = 0.64$  and  $R^2 = 0.45$  for 80 and 95°C, respectively) were fitted to the data.

decrease in E was more pronounced as the duration of the heat treatment increased at both 80 and 95°C (fig. 3). Across all levels of heating time, E values of films heated at 80°C were not significantly ( $P > 0.05$ ) different than E values of films heated at 95°C.

Similar to the results of the present study, increases in film TS and decreases in film E also were observed for wheat gluten protein films heat-treated at 80°C (Kolster et al., 1992). The effect of heating on film TS and E values can be partially attributed to the development of heat-induced cross-linking within the film structure. According to Cheftel et al. (1985), thermal treatments of proteins at alkaline pH (such as the pH of SPI films in the present study) promote formation of intra- and intermolecular cross-links. Such cross-links result from condensation of lysine, cysteine, or ornithine amino acid residues with a residue of dehydroalanine, the latter being formed by a  $\beta$ -elimination reaction starting with cysteine or phosphoserine residues (Cheftel et al., 1985). Presumably, such cross-links contributed to the increased toughness (higher TS) and reduced flexibility (lower E) of heated SPI films.

Besides heat-induced cross-linking, differences in MC also were considered as contributing factors to the observed increases in TS and decreases in E of heated SPI films. It has been shown that water functions as a plasticizer of wheat gluten and corn zein protein films reducing film TS and increasing film E (Gennadios et al., 1993b; Gontard et al., 1993). As mentioned earlier, the MC of SPI films (stored at conditions similar to those of conditioning films prior to tensile testing) decreased with heating time. As a result, the plasticizing effect of water was lessened as the duration of film heat treatment increased.

#### WATER SOLUBILITY

Heat-treated SPI films had substantially lower WS values (fig. 4) than the WS of  $17.2 \pm 1.2\%$  recorded for unheated films. Across all heating times, films heated at 95°C had significantly ( $P < 0.05$ ) lower (by  $5.4 \pm 0.3\%$ ) WS values than films heated at 80°C.

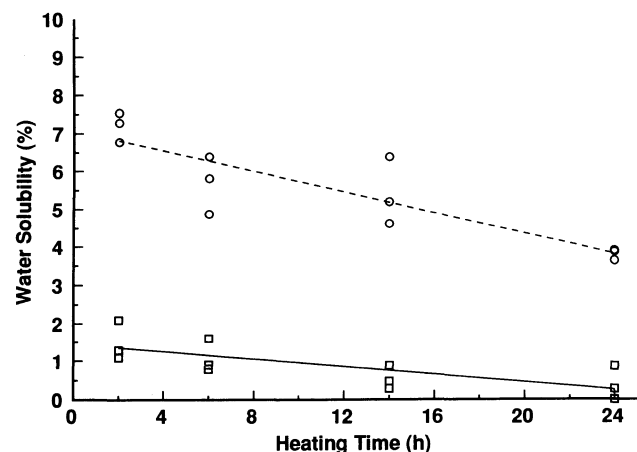


Figure 4—Water solubility values of soy protein isolate films heated at 80°C (○ with dashed line) and 95°C (□ with solid line). Linear regression lines ( $R^2 = 0.77$  and  $R^2 = 0.55$  for 80 and 95°C, respectively) were fitted to the data.

The drastic reduction of WS for most proteins subjected to heat treatment is well documented in the literature (Cheftel et al., 1985). Reductions in WSs following heat treatments have been reported for corn zein, gelatin, and fibrin protein films (Howland, 1961; Howland and Reiners, 1962; Julius, 1967).

#### WATER VAPOR PERMEABILITY

The WVP values, along with actual RH conditions at the undersides of films during testing, of control and heated SPI films are presented in table 1. A reduction in film WVP was evidenced as heating times increased for both 80 and 95°C. It also was noticed that the actual RH at the inner film surface, and, therefore, the RH gradient applied across the films during testing, increased with the severity of the heat treatment. Therefore, the observed differences in WVP values of SPI films would have been even greater under equal RH gradient conditions across the films. Films heated at 95°C had significantly ( $P < 0.05$ ) lower WVP than films heated at 80°C for 2 and 6 h of heating time. For heating times of 14 and 24 h, films heated at 95°C had lower, but not significantly ( $P > 0.05$ ), WVP mean values than films heated at 80°C. The decrease in WVP of heated films can be attributed to: 1) formation of covalent links within the films during heating; and 2) lower WS of heated films, an indication of a decrease in protein hydrophilicity.

#### COLOR

Color characteristics of films can be important factors for consumer acceptance of such films in edible or nonedible packaging applications. The Hunter L, a, and b color values and the total color difference ( $\Delta E$ ) values of control and heated SPI films are presented in table 2. The main difference between heated and unheated control films was the increased positive b values (yellowness) of heated films. The length of heating did not seem to be important to the film color; however, the temperature of heating was important. Films heated at 95°C had greater ( $P < 0.05$ ) positive b (yellowness) and negative a (redness) values, and, consequently, higher  $\Delta E$  values than films heated at 80°C. It is noted that color measurements of films may be affected by film moisture content and thickness. For example, films thicker than the ones prepared in this study ( $96.5 \pm 4.6 \mu\text{m}$ ) would appear more yellowish (higher positive b values).

Table 1. Effect of heating at 80 and 95°C on WVP of soy protein isolate films ( $96.5 \pm 4.6 \mu\text{m}$  in thickness) calculated using the ASTM E 96-80 Method at 25°C

Heating Time (h)	80°C		95°C	
	WVP* $\times 10^{-9}$ (g·m/m <sup>2</sup> ·s·Pa)	RH Inside Cup† (%)	WVP* $\times 10^{-9}$ (g·m/m <sup>2</sup> ·s·Pa)	RH Inside Cup† (%)
0	3.61 $\pm$ 0.27a	67.22 $\pm$ 0.96	3.61 $\pm$ 0.27a	67.22 $\pm$ 0.96
2	3.12 $\pm$ 0.28b	69.14 $\pm$ 0.85	2.49 $\pm$ 0.23c	70.94 $\pm$ 1.10
6	2.64 $\pm$ 0.28c	69.91 $\pm$ 0.42	1.91 $\pm$ 0.14d	72.68 $\pm$ 0.17
14	2.00 $\pm$ 0.25d	73.62 $\pm$ 0.11	1.71 $\pm$ 0.05d	74.46 $\pm$ 0.19
24	1.73 $\pm$ 0.17d	74.45 $\pm$ 0.10	1.64 $\pm$ 0.05d	75.25 $\pm$ 0.06

\* WVP values are means of three replicates plus/minus a standard deviation. Any two WVP means followed by the same small-case letter are not significantly ( $P > 0.05$ ) different according to Duncan's multiple range test.

† Actual RH values (means of three replicates plus/minus a standard deviation) at the underside of films calculated as described by Gennadios et al. (1994c) to account for resistance of stagnant air layer between film and water surface in testing cups. RH outside cups was 50%.

Table 2. Hunter color values (L, a, and b) and total color difference ( $\Delta E$ ) for soy protein films heated at 80 and 95°C for various times\*†

Heating Time (h)	L	a	b	$\Delta E$
Control				
0	93.22 ± 0.50ab	-2.50 ± 0.09a	13.55 ± 0.14d	12.38 ± 0.13d
80°C				
2	92.94 ± 0.13abc	-2.69 ± 0.09abc	14.89 ± 0.95d	13.75 ± 0.94d
6	92.88 ± 0.48abc	-2.59 ± 0.20ab	15.43 ± 0.69cd	14.26 ± 0.77cd
14	93.30 ± 0.35a	-2.46 ± 0.10a	15.08 ± 0.25d	13.78 ± 0.34d
24	93.34 ± 0.06a	-2.44 ± 0.05a	15.41 ± 0.66cd	14.09 ± 0.66cd
95°C				
2	92.41 ± 0.12bcd	-3.03 ± 0.12d	17.78 ± 0.48bc	16.68 ± 0.42bc
6	92.35 ± 0.60cd	-2.97 ± 0.09d	19.53 ± 1.90ab	18.36 ± 1.96ab
14	92.77 ± 0.08abcd	-2.80 ± 0.13bcd	18.82 ± 0.94b	17.55 ± 0.90b
24	92.03 ± 0.70d	-2.92 ± 0.23cd	21.67 ± 2.91a	20.47 ± 2.99a

\* Film color was measured after placing films on top of a standard white plate with Hunter color values of L = 96.86, a = -0.02, and b = 1.99.

† Presented values are means of three replicates plus/minus a standard deviation. Any two means in the same column followed by the same small-case letter are not significantly different ( $P > 0.05$ ) according to Duncan's multiple range test.

## CONCLUSIONS

Properties of SPI films can be substantially modified by heat treatments, thus, helping tailor such films to specific packaging applications. Increased TS and reduced WS and WVP are all desirable heat-induced improvements of SPI film properties. However, the above property improvements come at the expense of decreases in film E (flexibility) and increased yellow color.

**ACKNOWLEDGMENT.** We acknowledge partial funding from the Nebraska Soybean Board.

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