Soy Protein Biopolymers Cross-Linked with Glutaraldehyde

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ABSTRACT: Biopolymers from soy protein isolate (SPI) cross-linked with glutaraldehyde (GA) were prepared. Surface hydrophobicities of SPI–GA biopolymers and SPI were 4.4 and 11.5, respectively. The solubility profile of SPI was slightly higher than that of SPI–GA biopolymers. Foaming capacities of SPI–GA biopolymers (23 mL) were higher than that of SPI (19 mL), but similar to egg white (22 mL). Foaming stabilities of SPI–GA biopolymers (120 min) were significantly higher than those of SPI (40 min) and egg white (98 min). The emulsifying properties of SPI–GA biopolymers were lower than those of SPI and bovine serum albumin ($P > 0.05$). Tensile strength (TS) and elongation at break (ETB) of SPI–GA biopolymer films were significantly higher than those of glycerol-plasticized soy protein films. TS and ETB of SPI–GA biopolymer films increased with increasing GA concentrations. GA treatment intensified yellowness of SPI–GA biopolymer films. SPI–GA biopolymers may have potential use for biodegradable packaging materials.

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KEY WORDS: Biopolymers, cross-linking, glutaraldehyde, protein films, soy protein isolate, tensile strength.

Because of their structural characteristics, soy proteins have potential for industrial applications including use in plastics, adhesives, films, packaging materials, and reinforced composite materials. Expanded utilization of soy proteins is limited owing to strong competition from synthetic petroleum-based products. However, as a result of increasing concern over environmental safety of nondegradable synthetic products, there is interest in natural degradable products from renewable sources as alternatives to synthetic polymers. Soy protein is a viable renewable resource for producing environmentally safe industrial products. The potential for use of biodegradable soy protein films as packaging materials depends on their mechanical and barrier properties. Although soy protein has film-forming properties, the films have poor mechanical and moisture barrier properties. Modification of soy proteins to obtain desired physical properties and understanding of the basic structural changes of the modified proteins are extremely important to produce films with improved mechanical and barrier properties.

Glutaraldehyde (GA) is well known for its ability to react with protein and to produce cross-linked polymers (1). The ability of mono- and bi-functional aldehydes to promote covalent intermolecular and intramolecular cross-linking of protein is well documented (1–3). The sites of cross-linking between aldehydes and proteins are likely lysine and histidine basic groups, but reaction of aldehydes with sulfhydryl groups of cysteine is also possible (3,4). Aldehydes have been utilized for tanning collagen in the production of leather (5) and for sausage casings (6). Ghorpade *et al*. (7) reported that cross-linking soy proteins with formaldehyde resulted in two-fold increases in tensile strength (TS) and puncture strength of soy protein films.

To exploit the benefit of soy proteins fully and to obtain desirable physicochemical properties for biodegradable film application, modification of protein structural characteristics is necessary. The objective of this study was to produce soy protein isolate–glutaraldehyde (SPI–GA) biopolymers with enhanced functional properties and biodegradable films by chemical modification.

MATERIALS AND METHODS

Materials. Commercial defatted soy flour with protein dispersibility index (PDI) of 70 was supplied by Archer Daniels Midland Co. (Decatur, IL). Bovine serum albumin (BSA), egg white (EW), and GA were purchased from Sigma Chemical Co. (St. Louis, MO). Glycerin as a plasticizer was purchased from Fisher Scientific Co. (Pittsburgh, PA). All other reagents were of analytical reagent grade and purchased from Fisher Scientific and Sigma Chemical Co.

Preparing biopolymers using GA cross-linking. SPI with a PDI of 70 was prepared using the method of Wolf and Cowan (8) by extracting with alkali (pH 9.0) followed by acid (pH 4.5) precipitating, washing, and spray drying. The protein content of SPI determined by the micro-Kjeldahl method was 92%. The procedure to produce biopolymers consisted of adding 3.5 mL of 2.5% GA into 150 mL of 10% SPI solution (wt/vol, in 0.1 M phosphate buffer, pH 7.5) and stirring for 30 min. The mixture was kept at room temperature (25°C) for 12 h, and then freeze-dried.

Electrophoresis. The molecular weights of SPI–GA biopolymer were measured by sodium dodecyl sulfate-polyacryl-

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amide gel electrophoresis (SDS-PAGE) using a Bio-Rad Mini-Protean II electrophoresis system (Bio-Rad Laboratory, Richmond, CA) at a constant voltage of 200 V for about 45 min. Molecular weights (MW) of the protein bands were estimated by means of the SDS-PAGE protein standards, high MW range (Bio-Rad Laboratory).

Surface hydrophobicity. Surface hydrophobicity of protein was determined by using the 1-anilino-8-naphthalene sulfonate (ANS) binding method (9). Each protein sample was prepared at four concentrations: 1.9×10^{-3}, 3.8×10^{-3}, 7.5×10^{-3}, and 1.5×10^{-2}% (wt/vol, protein basis, in 0.01 M phosphate buffer, pH 7.0). In this range, a linear relationship between fluorescence intensity and SPI concentration was observed ($R^2 = 0.99$). Twenty microliters of 8 mM ANS in 0.01 M phosphate buffer (pH 7.0) was added to each 4.0 mL protein solution. Fluorescence intensity was recorded at 390 nm excitation and 470 nm emission using a Kontron Spectrofluorometer, model SFM23/B (Kontron Ltd., Zurich, Switzerland). The slope of the plot of fluorescence intensity vs. protein concentration, which was calculated by linear regression ($R^2 = 0.99$), was used as an index of surface hydrophobicity.

Protein solubility. Protein and biopolymer solutions (1%, wt/vol) were prepared. The pH values of the solutions were adjusted to 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0, respectively. Each solution was stirred for 30 min at room temperature and centrifuged at $10{,}000 \times g$ for 10 min. Supernatant (1 mL) was added to 4 mL of burette reagent. Absorbance was measured at 540 nm after 15 min incubation at room temperature. The standard curve was prepared by using SPI at pH 12.0 (at this pH, SPI is completely dissolved).

Thermal properties. Differential scanning calorimetry (DSC) was performed with a PerkinElmer DSC Pris I analyzer (Norwalk, CT). Protein samples were dissolved in a 60 mM phosphate buffer (pH 7.0) containing 100 mM NaCl at 40–60 mg/mL. Forty-five mL of each sample was sealed in a stainless steel pan and heated from 45 to 130°C at a rate of 10°C/min against another pan containing 45 mL of buffer without protein as a reference. Denaturation temperature and enthalpy of samples were calculated using software for thermal analysis data.

Emulsifying properties. Emulsifying activity and stability were determined by the turbidimetric method of Pearce and Kinsella (10). Portions of emulsion (50 µL) were pipetted from the bottom of the container into 5 mL of 0.1% SDS (wt/vol) solution immediately (0 min) and 10 min after homogenization. Absorbance of the SDS solution was measured at 500 nm using a double-beam spectrophotometer. Absorbance at zero time ($t = 0$ min) was expressed as emulsifying activity of protein, and emulsifying stability (ES) was calculated by using the equation

$$ES = T_0 \times t/T' \quad [1]$$

where T_0 is the turbidity at 0 min after homogenization, T' is the change in turbidity between 0 and 10 min, and t is the time interval between 0 and 10 min.

Foaming properties. Foaming capacity was determined by measuring the volume of foams after the introduction of air (90 cm^3/min) for 15 s into 2.5 mL of 1% protein solution (wt/vol, in 0.01 M phosphate buffer, pH 7.4) in a 25-mL graduated cylinder. Foaming stability (FS) was calculated by using the following equation:

$$FS = V_0 \times t'/V' \quad [2]$$

where V_0 is the foam volume at 0 time, t' is the amount of time for the observation occurring during the interval (30 min), and V' is the change in the foam volume (11).

Preparation of films. To determine the effect of GA concentration, GA (0.1, 0.2, 0.3, and 0.4 g) was added into soy protein film-forming solution (5 g/100 mL water). Glycerin (50% w/w of protein) was added to film-forming solutions to ensure film plasticization. The pH of film-forming solutions was adjusted to 9.0 with 1.0 N NaOH. The mixtures were stirred for 30 min and then heated at 85°C for 30 min. These solutions were poured onto plastic plates and dried overnight at room temperature (22–25°C). For the control film, SPI (5 g) was dispersed in 95 mL water, and plasticizer (glycerol, 2.5 g) was added.

Mechanical properties of films. TS of protein films were measured according to standard method D882-91 (12) using a Texture Analyzer (TA.XT2; Texture Technologies Corp., Scarsdale, NY). Film samples were conditioned at ambient temperature and 50% relative humidity for at least 48 h prior to textural analyses. TS measurements were performed by mounting film strips (40×5.5 mm) on the texture analyzer. Initial grip separation was 35 mm and cross-head speed was set at 2 mm/s in a tension mode. TS in MPa was computed as peak force divided by cross-sectional area of specimen. Elongation at break (ETB) was the dimensionless measure of a film's ability to stretch. The percent change in length was experienced by a material due to pulling stress before breakage.

Film color. Hunter color of films was measured using a Gardner Colorimeter (ColorGard System/05, Pacific Scientific, Silver Spring, MD). Sample specimens were placed on the surface of a white standard plate (Calibration Plate White-1415) and Hunter L, a, and b color values were measured (13). The ranges of the three color coordinates were 0 black to 100 white, − greenness to + redness, and − blueness to + yellowness, respectively. Standard values refer to the white calibration plate (L = 94.47, a = –0.81, b = –0.86).

Statistical analysis. Three replications were performed in a completely randomized design. Data were analyzed using the general linear model procedures of SAS (14) to determine differences between treatment means. Pair-wise comparison of all treatment means was performed using the least significant difference procedure with significance defined at $P < 0.05$.

RESULTS AND DISCUSSION

Molecular weights. The molecular weights of soy protein polymers prepared by GA cross-linking are reflected in Figure 1. GA was used as a cross-linker for biopolymers in which

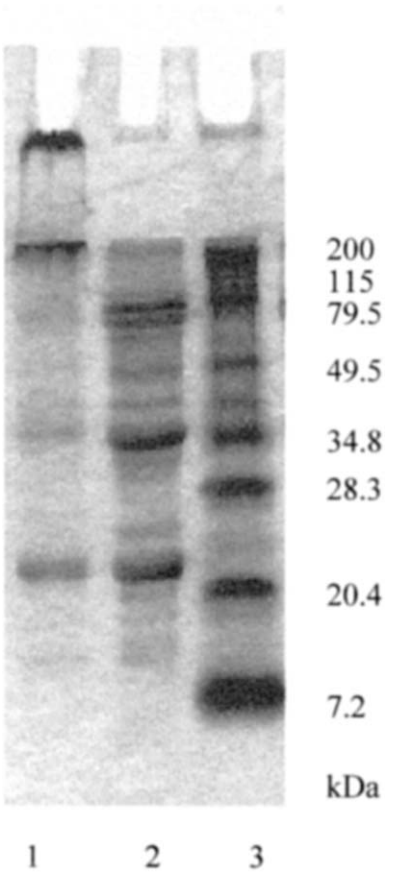

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns of soy protein isolate (SPI)-glutaraldehyde (GA) polymer and SPI. Lane 1 is the SPI–GA biopolymer, lane 2 is the SPI, and lane 3 is the molecular weight standard.

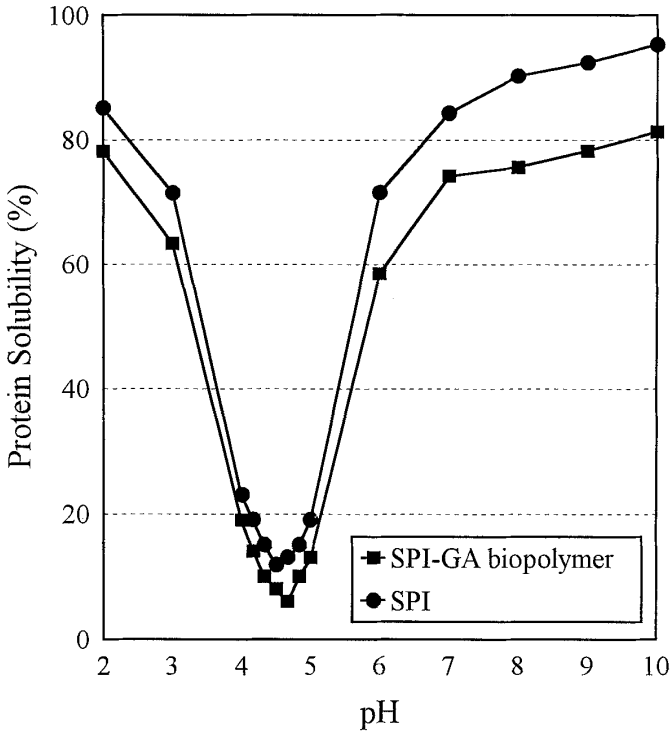

FIG. 2. Protein solubilities and pH of SPI–GA biopolymer and SPI. Means of three values ($P > 0.05$). For abbreviations see Figure 1.

ε-amino groups of lysine in soy proteins are expected to form a Schiff base with GA. The reaction of SPI with GA introduces a "chemical cross-link" to soy protein, which makes the soy protein insoluble in water. The molecular weights of SPI–GA biopolymer prepared by cross-linking SPI with GA were estimated above 200 kDa. The increased molecular weight indicated that the formation of soy protein polymers was due to intermolecular cross-linking between soy protein molecules by GA. The cross-linking reaction of protein can take place intermolecularly (between molecules) and/or intramolecularly (within molecules). However, only intermolecular cross-linking leads to increased molecular weight.

Surface hydrophobicity. Surface hydrophobicities of SPI–GA biopolymers, SPI, and BSA were 4.4, 11.5, and 90, respectively. The surface hydrophobicities of SPI–GA biopolymers were significantly lower than that of SPI ($P > 0.05$). This low hydrophobicity was probably due to cross-linking of protein molecules that altered the structure of soy protein and reduced the number of hydrophobic amino acids exposed to solvent.

Protein solubility. Protein solubility profiles of SPI–GA biopolymer and SPI are given in Figure 2. The solubility curve of SPI was higher than that of SPI–GA biopolymer. The minimum solubilities of SPI were observed at pH 4.5, indicating that this pH was the isoelectric point of SPI. GA cross-linking leads to a change in isoelectric point due to the change of surface charges of protein. GA has been used as a cross-linker for biopolymers in which ε-amino acid groups of lysine in protein were expected to form a Schiff base with GA. Slight increases in the solubility in the isoelectric point region were observed with SPI–GA biopolymer. Hence, the isoelectric point of SPI was shifted by GA cross-linking.

Thermal properties. DSC thermograms of SPI–GA biopolymer and SPI are given in Figure 3. For SPI–GA biopolymers, the denaturation temperature of 7S (β-conglycinin) fraction at 80°C was absent, and a new denaturation temperature was observed at 110°C with enthalpy of 4.5 J/g protein. Although denaturation temperature of the 11S (glycinin) fraction at 95°C was the same as that of SPI, enthalpy decreased to 2.1 J/g protein. Decreased denaturation enthalpy of 11S fraction in SPI–GA biopolymer was evidence of partial denaturation of 11S fraction. These results suggest that alteration of soy protein conformation could be changed by GA cross-linking. The denaturation peaks at 97 and 110°C resulted from 7S and 11S after cross-linking. The thermal stabilities of SPI–GA were higher than that of SPI, since the conformational structures of SPI–GA biopolymer were stable up to 95°C. The increase in molecular weight of SPI–GA biopolymer may increase its denaturation temperatures.

Foaming properties. Foaming properties of SPI–GA biopolymers, SPI, and EW are given in Figure 4. The foam-

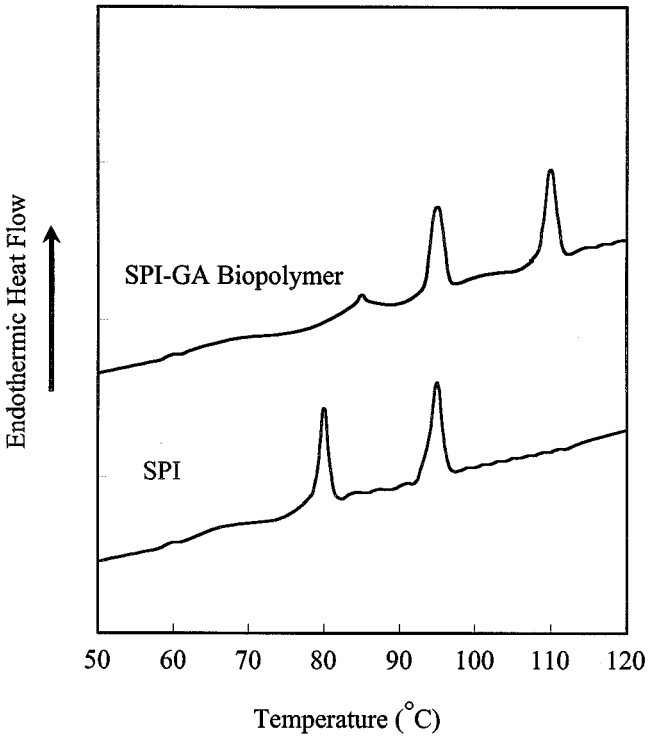

FIG. 3. Differential scanning calorimetry thermograms of SPI–GA biopolymer and SPI. For abbreviations see Figure 1.

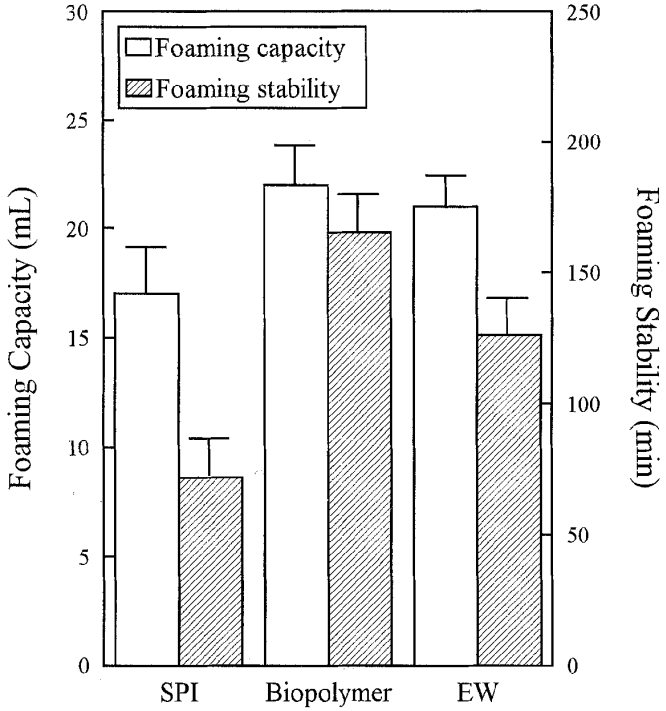

FIG. 4. Foaming properties of SPI–GA biopolymer, SPI, and egg white (EW). Means of three values ($P > 0.05$). Foaming stability was calculated by Equation 2. For abbreviations see Figure 1.

ing capacities of SPI–GA biopolymers (23 mL) were higher than that of SPI (19 mL) but were similar to EW (22 mL) ($P > 0.05$). Foaming stabilities of SPI–GA biopolymers, SPI, and EW were 120, 40, and 98 min, respectively. Foaming stabilities of SPI–GA biopolymers were significantly higher than those of SPI and EW ($P > 0.05$). The formation of protein-based foams involves the diffusion of proteins toward the air-water interface and rapid conformational change and rearrangement at the interface. However, foam stability requires a thick, cohesive, continuous, air-permeable protein film around each gas bubble (15). Increased foaming capacity and stability might be due to increased molecular size of SPI–GA biopolymers, since large molecular weight proteins produce thick adsorbed films with good surface rheological properties (16). In addition, elimination of charged amino ε-groups of lysine and histidine residues by cross-linking could have enhanced foam stability (17).

Emulsifying properties. The emulsifying properties of SPI–GA biopolymers, SPI, and BSA are given in Figure 5. The emulsifying activities of SPI–GA biopolymers (0.382) were lower than that of SPI (0.55) and BSA (1.111) ($P > 0.05$). The emulsion stabilities of SPI–GA biopolymers (20 min) were lower than those of SPI (22 min) and BSA (25 min) ($P > 0.05$). The lower emulsifying activities and stabilities of SPI–GA biopolymers were probably due to their low surface hydrophobicity compared with SPI and BSA. Generally, emulsifying activity of protein positively correlated with its surface hydrophobicity.

Mechanical properties of films. The effect of GA concentration on mechanical properties of soy protein films is given in Table 1. TS values of SPI–GA films were significantly higher than that of control soy protein film ($P < 0.05$). TS of

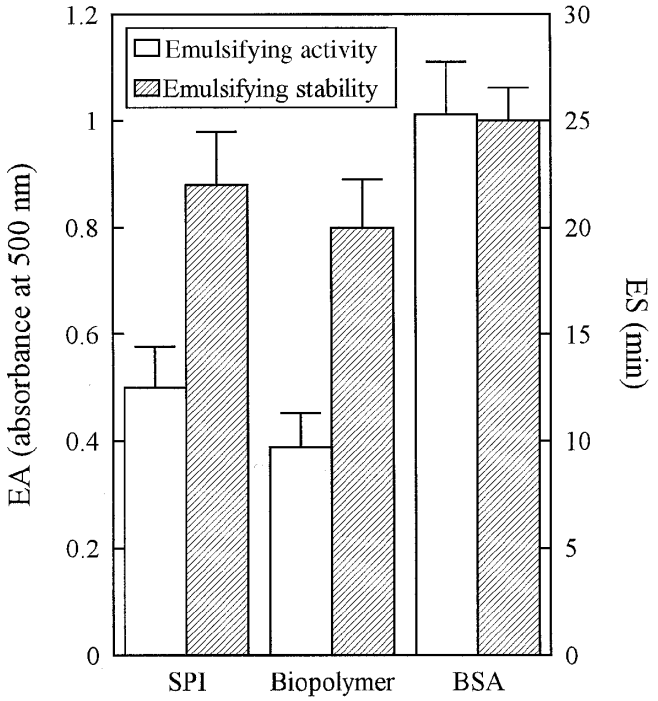

FIG. 5. Emulsifying activity (EA) and emulsion stability (ES) (Eq. 1) of SPI–GA biopolymer, SPI, and bovine serum albumin (BSA). Means of three values ($P > 0.05$). For abbreviations see Figure 1.

TABLE 1
Effect of Glutaraldehyde on Mechanical Properties of Soy Protein Isolate Films[a]

Glutaraldehyde (w/w of protein)	Tensile strength (MPa)	Elongation at break (%)
Control	$8.32^{c} \pm 1.34$	$38.71^{d} \pm 1.18$
0.1	$12.24^{b} \pm 0.59$	$43.75^{c} \pm 2.12$
0.2	$13.66^{b} \pm 0.36$	$61.01^{b} \pm 1.32$
0.3	$14.06^{a} \pm 0.87$	$67.18^{b} \pm 1.77$
0.4	$14.89^{a} \pm 0.51$	$71.25^{a} \pm 0.70$

[a]Values within each column with the same roman superscript are not significantly different ($P < 0.05$).

TABLE 2
Hunter Color Values (L, a, and b) and Total Color Difference (ΔE) of Soy Protein Films Cross-Linked with Glutaraldehyde (GA)[a]

GA (%)	L	a	b	ΔE
0	$53.11^{a} \pm 1.34$	$-3.64^{c} \pm 0.11$	$6.75^{c} \pm 0.12$	$41.15^{c} \pm 1.34$
0.1	$46.35^{b} \pm 1.17$	$-4.23^{b} \pm 0.26$	$7.23^{b} \pm 0.11$	$48.92^{b} \pm 0.36$
0.2	$46.29^{b} \pm 1.61$	$-4.51^{b} \pm 0.34$	$7.32^{b} \pm 0.21$	$49.01^{b} \pm 0.54$
0.3	$42.77^{c} \pm 1.47$	$-5.62^{a} \pm 0.14$	$7.64^{a} \pm 0.28$	$52.61^{a} \pm 1.18$

[a]Values within each column with the same roman superscript are not significantly different ($P < 0.05$).

SPI–GA films increased with increasing concentrations of GA. ETB of SPI–GA films were also higher than those of the control soy protein films ($P > 0.05$). These data suggest that GA cross-linking enhanced mechanical properties (TS and elongation) of soy films because of the covalent intermolecular and intramolecular cross-linking of protein.

Hunter L, a, and b color values and total color differences (ΔE) for films are shown in Table 2. SPI–GA biopolymer films at pH 9.0 showed the higher positive b (yellowness) values. SPI–GA biopolymer films were generally clearer (highest L value) and more uniform. The effect of GA on Hunter color values of SPI–GA biopolymer films at 0.1, 0.2, and 0.3% (w/w of total soy protein) was substantial. Increasing GA concentration resulted in decreased L and increased b (yellowness).

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