Solubility, Tensile, and Color Properties of Modified Soy Protein Isolate Films†

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Protein solubility (PS) values of different soy protein isolate (SPI) films were determined in water, 0.01 N HCl, 0.01 N NaOH, 4 M urea, and 0.2 M 2-mercaptoethanol. Tensile and color (*L*, *a*, and *b* values) properties of films also were determined. Control films were cast from heated (70 °C for 20 min), alkaline (pH 10) aqueous solutions of SPI (5 g/100 mL of water) and glycerin (50% w/w of SPI). Additional films were cast after incorporation of dialdehyde starch (DAS) at 10% w/w of SPI or small amounts of formaldehyde in the film-forming solutions. Also, control film samples were subjected to heat curing (90 °C for 24 h), UV radiation (51.8 J/m^2), or adsorption of formaldehyde vapors. PS of control films was highest ($P < 0.05$) in 2-mercaptoethanol, confirming the importance of disulfide bonds in SPI film formation. All treatments were effective in reducing ($P < 0.05$) film PS in all solvents. Both DAS and adsorbed formaldehyde rendered the protein in films practically insoluble in all solvents. Adsorption of formaldehyde vapors and heat curing also substantially increased ($P < 0.05$) film tensile strength from 8.2 to 15.8 or 14.7 MPa, respectively. However, heat curing decreased ($P < 0.05$) film elongation at break from 30 to 6%. Most treatments had small but significant ($P < 0.05$) effects on *b* color values, with DAS-containing films having the greatest ($P < 0.05$) mean *b* value (most yellowish). Also, DAS-containing, heat-cured, and UV-irradiated films were darker, as evidenced by their lower ($P < 0.05$) *L* values, than control films. It was demonstrated that PS of SPI films can be notably modified through chemical or physical treatments prior to or after casting.

Keywords: *Protein films; soy protein; dialdehyde starch; formaldehyde; cross-linking*

INTRODUCTION

Soy protein is an abundant side-product of the soybean oil industry. Production of edible/biodegradable films or coatings has the potential to add value to soy protein. The film-forming ability of soy protein has long been noted. Two types of soy protein-based films have been discussed in the literature: films (known as "yuba") formed on the surface of heated soy milk (Gennadios and Weller, 1991) and free-standing films cast from soy protein solutions (Brandenburg et al., 1993; Gennadios et al., 1993; Kunte et al., 1997). In general, soy protein films, similar to films from other proteins, provide limited resistance to water vapor transfer. This is attributed to the inherent hydrophilicity of proteins and the notable amounts of hydrophilic plasticizers incorporated into protein films (Krochta and De Mulder-Johnston, 1997).

Property modifications of soy protein-based films through physical (Gennadios et al., 1996, 1998a; Rangavajhyala et al., 1997), chemical (Ghorpade et al., 1995; Rhim, 1998; Rhim et al., 1998, 1999a), or enzymatic (Motoki et al., 1987; Stuchell and Krochta, 1994; Yildirim and Hettiarachchy, 1997) treatments, which may render the films inedible, have been investigated. Such treatments aimed to promote cross-linking within the protein film network. The effectiveness of cross-linking treatments can be determined by monitoring reductions in the solubility of the films' protein. Our main objective was to investigate the effects of selected physical and chemical treatments on the protein solubility in various solvents of soy protein isolate (SPI) films. Film tensile and color properties also were determined.

MATERIALS AND METHODS

Film Preparation. Film-forming solutions were prepared by slowly dissolving 5 g of SPI (minimum 90% protein content on dry basis, SUPRO 620, Protein Technologies International, St. Louis, MO) in a constantly stirred mixture of distilled water (100 mL) and glycerin (2.5 g). Glycerin was added as a plasticizer to overcome film brittleness and to obtain free-

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standing films. The solution pH was adjusted to 10 ± 0.1 with 1 N sodium hydroxide. Alkaline conditions favor SPI film formation, presumably by aiding protein dispersion in film-forming solutions (Okamoto, 1978; Gennadios et al., 1993). After heating for 20 min at 70 °C in a constant-temperature water bath, the solutions were strained through eight layers of cheesecloth (grade 40, Fisher Scientific, Pittsburgh, PA) to remove any small lumps (minuscule amounts) and cast onto leveled, Teflon-coated glass plates (21 cm × 35 cm). Casting of SPI films on Teflon-coated surfaces was previously practiced (Jaynes and Chu, 1975; Gennadios et al., 1993), although high-density polyethylene-coated plates also were used for the same purpose (Stuchell and Krochta, 1994). Film thickness was controlled by casting the same amount (80 mL) of film-forming solution per plate. The castings were dried at ambient conditions (25 °C) for ~20 h. Dried films were peeled from the plates, and specimens, for property testing, were cut (2 cm × 2 cm for protein solubility; 2.54 cm × 10 cm for tensile testing; and 7 cm × 7 cm for color measurements). In addition to "control" films prepared in this manner, additional films were prepared following the treatments described below.

Heat Curing. Control film specimens were mounted on glass plates by applying masking tape around the film edges and heated at 90 °C for 24 h in an air-circulating oven (Gennadios et al., 1996). The masking tape held film specimens flat, thus preventing "curling" during heating.

UV Irradiation. Control film specimens were irradiated for 24 h in a metal, light-tight cabinet equipped with a 253.7 nm UV lamp (Gennadios et al., 1998a). The UV light intensity at the cabinet center was 0.6 mW/m^2, and film specimens received a UV radiation dosage of 51.8 J/m^2 over 24 h of exposure.

Adsorption of Formaldehyde. Control film specimens were placed in a vacuum desiccator over 500 mL of formaldehyde solution (37% w/w in water) for 2 h at ambient temperature (Rhim, 1998). The desiccator was saturated with formaldehyde vapors for 2 h prior to the introduction of the film samples.

Incorporation of Dialdehyde Starch. Films were prepared as described above after dialdehyde starch (DAS) (81.8% starch oxidation; Sigma, St. Louis, MO) was added to control film-forming solutions at 10% w/w of SPI (Rhim et al., 1998).

Incorporation of Formaldehyde. Direct addition of formaldehyde solution (37% w/w) to SPI film-forming solutions has been reported (Ghorpade et al., 1995). However, in such cases, the viscosity of the film-forming solutions increases rapidly as formaldehyde and protein interact. This can hinder casting and film formation. Therefore, minor amounts of formaldehyde were introduced into film-forming solutions as follows. A stock formaldehyde solution was prepared by adding 1 mL of concentrated formaldehyde (37% w/w in water) into 1000 mL of distilled water. Then, instead of using 100 mL of distilled water to prepare film-forming solutions, 30 mL of stock solution and 70 mL of distilled water or 50 mL of stock solution and 50 mL of distilled water were used. The cast films from such film-forming solutions were designated F30 or F50, respectively.

Film Thickness and Conditioning. Film thickness was measured to the nearest 2.54 μm (0.0001 in) with a hand-held micrometer (B. C. Ames Co., Waltham, MA). Five thickness measurements were taken on each tensile testing specimen along the length of the rectangular strip, and the mean value was used in tensile strength calculations. All film specimens were conditioned for 2 days in an environmental chamber (model RC-5492, Parameter Generation and Control, Inc., Black Mountain, NC) at 50% relative humidity (RH) and 25 °C before testing (ASTM, 1995).

Film Moisture Content. To avoid heat curing, film specimens were not subjected to oven-drying to determine their initial dry matter prior to testing. Instead, the initial dry matter of conditioned films was determined on different specimens (two from each film) by drying in an air-circulating oven (105 °C for 24 h).

Protein Solubility in Various Solvents. The soluble protein of films in water, acid, alkali, urea, and 2-mercaptoethanol (2-ME) was determined. Conditioned film samples (2 cm × 2 cm) were weighed (±0.0001 g) and transferred into glass test tubes with 10 mL of distilled water, 0.01 N hydrochloric acid (pH 2.06 ± 0.01), 0.01 N sodium hydroxide (pH 12.07 ± 0.01), 4 M urea, or 0.2 M 2-ME. The tubes were fitted with screw caps and mildly shaken for 12 h at ambient temperature (≈23 °C) using a reciprocating shaker (Eberbach Corp., Ann Arbor, MI). Following filtration with Whatman No. 4 filter paper (Whatman International Ltd., Maidstone, U.K.), protein concentration in the solvent was measured using a Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA) as described by Bradford (1976). Bovine serum albumin (98−99% albumin content, Sigma) was used as the standard for protein quantitation. Protein solubility (PS) was expressed in milligrams of solubilized protein per milliliter of solvent.

Tensile Testing. Film tensile strength (TS) and percentage elongation at break (E) were determined with an Instron Universal Testing Machine (model 5566, Instron Engineering Corp., Canton, MA). Initial grip separation was set at 50 mm, and cross-head speed was set at 50 mm/min. TS was calculated by dividing the maximum load by the initial cross-sectional area of the specimen (ASTM, 1995). E was calculated by dividing the extension at rupture of the specimen by the initial gage length of the specimen (50 mm) and multiplying by 100 (ASTM, 1995).

Color Measurements. Color values of the films were measured with a portable colorimeter (CR-300 Minolta Chroma Meter, Minolta Camera Co., Osaka, Japan). Film specimens were placed on a white standard plate (calibration plate CR-A43; $L = 96.86$, $a = -0.02$, and $b = 1.99$) and the L, a, and b color values were measured. L values range from 0 (black) to 100 (white); a values range from −80 (greenness) to 100 (redness); and b values range from −80 (blueness) to 70 (yellowness).

Statistical Analysis. PS, TS, E, and color values for each type of film were determined in triplicate with individually prepared and cast films as the replicated experimental units. Statistics on a completely randomized design were determined using the General Linear Model procedure in SAS software (release 6.08, SAS Institute Inc., Cary, NC). Significantly ($P < 0.05$) different means were separated with Duncan's multiple-range test.

RESULTS AND DISCUSSION

Control Films. PS of control SPI films was substantially greater ($P < 0.05$) in 2-ME than in water or in other solvents (Table 1). This was expected because covalent disulfide (S−S) bonds are considered to be important in film formation by animal and plant proteins, such as soy protein, that contain cysteine/cystine amino acids (Okamoto, 1978; Gennadios et al., 1994; Krochta and McHugh, 1996; Handa et al., 1999; Were et al., 1999). In particular, the tendency of 7S and 11S soy protein fractions to polymerize through S−S bonds has been documented (Fukushima and Van Buren, 1970; Hoshi et al., 1982; Yamagishi et al., 1983). The 2-ME cleaved S−S bonds (Cheftel et al., 1985), thus increasing SPI film solubility.

Urea in concentrated aqueous solutions (4−8 M) disrupts hydrogen-bond interactions in proteins and decreases hydrophobic interactions by enhancing the solubility of hydrophobic amino acid residues in the aqueous phase (Cheftel et al., 1985). It has been reported that hydrophobic interactions also contribute to soy protein polymerization and insolubilization (Fukushima and Van Buren, 1970). However, the PS of control SPI films was lower ($P < 0.05$) in aqueous urea than in water (Table 1) due, most likely, to the predominance of hydrophilic amino acid residues in SPI. This indicated that S−S bonds played a far more important role in the SPI film structure than hydrophobic or hydrogen-bond

Table 1. Protein Solubility (Milligrams per Milliliter) in Various Solvents of SPI Films Subjected to Various Treatments[a]

film type[b]	water	0.01 N HCl	0.01 N NaOH	4 M urea	0.2 M 2-ME
control	0.36 ± 0.01[a]	0.22 ± 0.00[a]	0.47 ± 0.05[a]	0.22 ± 0.03[a]	0.72 ± 0.10[a]
heat cured	0.03 ± 0.01[e]	0.01 ± 0.00[d]	0.05 ± 0.01[d]	0.05 ± 0.01[d]	0.14 ± 0.03[d]
UV irradiated	0.11 ± 0.06[d]	0.09 ± 0.03[c]	0.26 ± 0.06[b]	0.05 ± 0.01[d]	0.16 ± 0.06[d]
FA adsorbed	ND	ND	ND	ND	ND
DAS added	0.01 ± 0.00[e]	ND	ND	ND	ND
FA added (F30)	0.27 ± 0.03[b]	0.11 ± 0.02[b]	0.28 ± 0.03[b]	0.18 ± 0.01[b]	0.46 ± 0.05[b]
FA added (F50)	0.22 ± 0.02[c]	0.10 ± 0.01[b]	0.16 ± 0.02[c]	0.12 ± 0.01[c]	0.35 ± 0.03[c]

[a] Means of three replicates ± standard deviations. Any two means in the same column followed by the same letter are not significantly ($P > 0.05$) different by Duncan's multiple range test. ND = not detected. [b] Refer to text for explanation of film treatments.

Table 2. Film Thickness (*D*), Moisture Content (MC), Tensile Strength (TS), and Elongation at Break (*E*) of SPI Films Subjected to Various Treatments[a]

film type[b]	*D* (μm)	MC (% wb)	TS (MPa)	*E* (%)
control	67.0 ± 1.1[d]	20.5 ± 1.0[e]	8.2 ± 0.2[d]	30.0 ± 3.3[cd]
heat cured	67.1 ± 1.6[d]	10.3 ± 0.1[f]	14.7 ± 0.4[b]	6.1 ± 0.7[e]
UV irradiated	67.0 ± 1.0[d]	21.7 ± 1.0[d]	10.0 ± 0.6[c]	23.3 ± 5.6[d]
FA adsorbed	67.1 ± 1.4[d]	23.3 ± 0.5[bc]	15.8 ± 0.1[a]	35.4 ± 7.9[c]
DAS added	76.1 ± 0.9[b]	25.3 ± 0.9[a]	8.0 ± 0.1[de]	46.7 ± 3.3[b]
FA added (F30)	68.3 ± 0.7[d]	22.3 ± 0.5[cd]	8.2 ± 0.2[d]	41.1 ± 6.9[bc]
FA added (F50)	71.7 ± 0.4[c]	22.2 ± 0.5[d]	8.3 ± 0.2[d]	100.1 ± 12.0[a]

[a] Means of three replicates ± standard deviations. Any two means in the same column followed by the same letter are not significantly ($P > 0.05$) different by Duncan's multiple range test. [b] Refer to text for explanation of film treatments.

interactions. Similarly, Gennadios et al. (1998b) reported that PS of egg white films was notably greater in urea/2-ME mixtures than in urea alone.

Similar to urea, the PS values of control SPI films were lower ($P < 0.05$) in the acidic medium (0.01 HCl) than in water (Table 1). In agreement, Sian and Ishak (1990) reported that the acid-sensitive soy protein forms insoluble complexes at pH ~2. In contrast, film PS in alkali was greater ($P < 0.05$) than the PS in water (Table 1). In general, soy proteins (and other seed proteins) are highly soluble at alkaline pH ~12 (Cheftel et al., 1985; Sian and Ishak, 1990).

Heat Curing. Thermal treatments of proteins promote formation of intra- and intermolecular cross-links, which mainly involve lysine and cystine amino acid residues (Cheftel et al., 1985). Improvements in film mechanical toughness and moisture barrier ability of cast, dried protein-based films through heat curing were reported (Weadock et al., 1984; Gennadios et al., 1996; Ali et al., 1997; Miller et al., 1997). Heat-cured SPI films had substantially lower ($P < 0.05$) PS in all solvents, including 2-ME, than control films (Table 1). This indicated that covalent cross-links, other than S−S bonds, were developed in heat-cured films. It is noted though that heat-cured films had lower ($P < 0.05$) moisture content (10.3% wet basis) than all other types of film (Table 2), although they were conditioned in the same manner. Perhaps the heat-induced cross-links enhanced film hydrophobicity, thereby decreasing moisture uptake during film conditioning. The lower moisture content could have contributed to the reduced PS of heat-cured films because water adsorption and film swelling probably took longer than in the case of the other films. Reduced PS values of SPI films due to heat curing also were reported earlier (Gennadios et al., 1996; Rangavajhyala et al., 1997). However, the SPI films discussed in the mentioned two studies had higher plasticizer contents (60 versus 50% glycerin w/w of SPI) than films in the present study. Also, PS measurement methodologies differed.

The reduced PS of heat-cured films was accompanied by increased ($P < 0.05$) TS and decreased ($P < 0.05$) *E* (Table 2) compared to control films. Presumably, heat-induced cross-linking contributed to increased strength (greater TS) and reduced extendibility (lower *E*) of films. Similar observations were previously reported for SPI films (Gennadios et al., 1996) and for films from wheat gluten (Ali et al., 1997) and whey protein (Miller et al., 1997). However, as mentioned, heat-cured films had lower moisture contents than the control films. Therefore, the increased TS and reduced *E* of the heat-cured films were partially attributed to their lower moisture content because water plasticizes hydrophilic protein-based films (Gontard et al., 1993; McHugh et al., 1994). In terms of color characteristics (Table 3), heat-cured films had slightly lower ($P < 0.05$) *L* values (decreased lightness) and notably greater ($P < 0.05$) *b* values (increased yellowness) than control films. This was in agreement with earlier findings by Gennadios et al. (1996). It also is possible that the lower moisture content contributed to the increased yellowness of heat-cured films.

Table 3. *L*, *a*, and *b* Color Values of SPI Films Subjected to Various Treatments[a]

film type[b]	*L*	*a*	*b*
control	93.0 ± 0.2[b]	−2.44 ± 0.05[d]	14.40 ± 0.27[d]
heat cured	91.6 ± 0.2[c]	−2.18 ± 0.03[b]	20.67 ± 0.38[b]
UV irradiated	91.7 ± 0.2[c]	−2.27 ± 0.07[c]	19.41 ± 0.20[c]
FA adsorbed	93.7 ± 0.2[a]	−1.08 ± 0.03[a]	10.03 ± 0.09[f]
DAS added	89.8 ± 0.3[c]	−1.04 ± 0.05[a]	25.96 ± 0.24[a]
FA added (F30)	92.6 ± 0.5[b]	−2.24 ± 0.06[bc]	13.78 ± 0.33[e]
FA added (F50)	92.9 ± 0.1[b]	−2.25 ± 0.04[bc]	13.67 ± 0.08[e]

[a] Means of three replicates ± standard deviations. Any two means in the same column followed by the same letter are not significantly ($P > 0.05$) different by Duncan's multiple range test. [b] Refer to text for explanation of film treatments.

UV Irradiation. UV radiation treatments of proteins are known to form cross-links, which mainly involve aromatic amino acid residues (Tomihata et al., 1992). Cross-linking of collagen films by UV radiation has been reported (Rubin et al., 1968; Weadock et al., 1984; Tomihata et al., 1992). More recently, UV treatments have been applied to cross-link protein films from SPI, wheat gluten, corn zein, casein, and egg albumen (Gennadios et al., 1998a; Rhim et al., 1999b). Film PS in all solvents decreased ($P < 0.05$) notably by UV

treatment (Table 1) compared to control films. The reduced PS in 2-ME suggested the occurrence of covalent bonds other than S−S bonds within the structure of the UV-treated films. In addition, the UV treatment increased ($P < 0.05$) film TS (Table 2) and darkened film appearance as evidenced by a lower ($P < 0.05$) *L* color value and a greater ($P < 0.05$) *b* color value (Table 3). Such darkening of UV-treated protein films also was previously observed (Gennadios et al., 1998a; Rhim et al., 1999b).

Addition of DAS. DAS is a polymeric aldehyde (molecular mass range 300−5000 kDa) prepared by reacting native starch with periodic acid (Mehltretter, 1963). The cross-linking effects of DAS on various proteins, such as collagen (Nayudamma, 1961), casein (Weakley et al., 1963), wheat gluten (Chatterji and Arnold, 1965), gelatin (Helmstetter, 1977), and corn zein (Spence et al., 1995), have been documented. Recently, DAS has been used to cross-link protein films from SPI (Rhim et al., 1998), egg white (Gennadios et al., 1998b), and corn zein (Parris et al., 1998). DAS addition at 10% w/w of SPI was highly effective in reducing ($P < 0.05$) film PS in all solvents (Table 1) compared to control films. It rendered the films practically insoluble. The insolubility of SPI−DAS films even in 2-ME suggested that cross-links other than S−S bonds had been formed within the film structure.

It was expected that such cross-linking would have resulted in increased film TS and decreased *E*. However, the DAS-containing films had similar ($P > 0.05$) TS and greater ($P < 0.05$) *E* than control films (Table 2). This was attributed to the higher ($P < 0.05$) moisture content of the films with DAS (Table 2) because, as mentioned, water plasticizes hydrophilic films. The greater moisture content of the DAS-containing films likely resulted from water binding by the large number of hydrophilic hydroxyl groups (one per repeating unit) on the DAS biopolymer. Similarly, increased moisture contents were previously reported for SPI-based films with various amounts of added DAS (Rhim et al., 1998). Films containing DAS had lower ($P < 0.05$) *L* color value and greater ($P < 0.05$) *b* color value (Table 3) than the other films. In general, the yellow/brown coloration associated with protein−aldehyde interaction is due to various intermediate or final products of the Maillard reaction (Cheftel et al., 1985). In particular, browning as a result of protein−DAS interactions was previously documented (Nayudamma et al., 1961; Spence et al., 1995; Gennadios et al., 1998b).

Formaldehyde (FA) Treatment. Low molecular mass aldehydes, such as FA, react with primary amino groups and sulfhydryl groups in proteins, forming intra- and intermolecular cross-links (Feeney et al., 1975). This has been utilized for the tanning of collagen in leather (Harlan and Feairheller, 1977). Ghorpade et al. (1995) reported that the addition of FA in SPI film-forming solutions resulted in films of increased TS and reduced water vapor permeability. FA also was used to cross-link collagen films (Lieberman and Gilbert, 1973), gelatin gels (Welz and Ofner, 1992), soft gelatin capsule shells (Hakata et al., 1994), and cottonseed protein films (Marquié et al., 1995, 1997). FA-treated SPI films were prepared either by allowing cast films to adsorb FA vapors or by directly incorporating small amounts of FA into the film-forming solutions. Films (FA30 and FA50) cast from solutions with added FA had reduced ($P < 0.05$) PS in all solvents compared to control films (Table 1). However, this treatment was not as effective as heat-curing, UV radiation, or reaction with DAS in reducing film PS. In contrast, films that were subjected to adsorption of FA vapors were practically insoluble in all solvents (Table 1).

FA30 and FA50 films cast from FA-containing SPI solutions did not differ ($P > 0.05$) from control films in terms of TS (Table 2). The FA50 films had greater ($P < 0.05$) *E* than all other films. However, the FA50 films were slightly thicker ($P < 0.05$) than control films (Table 2), and film thickness was not accounted for in *E* calculations. FA-adsorbed films had substantially greater ($P < 0.05$) TS than control films (Table 2) but similar ($P > 0.05$) *E* (Table 2). It was expected that the aldehyde-induced cross-linking within the film structure would also have reduced film *E*. Perhaps this discrepancy resulted from the higher ($P < 0.05$) moisture content of the FA-adsorbed films compared to the control films (Table 2) because, as mentioned, water can plasticize protein films and increase their extendibility. Color differences between control and FA30 or FA50 films were practically inconsequential (Table 3). The FA-adsorbed films had lower ($P < 0.05$) *b* color values (decreased yellowness) than all other film types.

Implication. It is evident from this study that the protein solubility characteristics of SPI films can be modified through treatments prior to or after casting. In particular, aldehydes appear to drastically reduce film solubility in an array of solvents. Modified SPI films of reduced solubility could find uses in mulching, renewable packaging, and other industrial applications.

LITERATURE CITED

Ali, Y.; Ghorpade, V. M.; Hanna, M. A. Properties of thermally-treated wheat gluten films. *Ind. Crops Prod.* **1997**, *6*, 177−184.

ASTM. Standard test methods for tensile properties of thin plastic sheeting. In *Annual Book of ASTM Standards*; American Society for Testing and Materials: West Conshohochen, PA, 1995; Vol. 8.01, pp 182−190.

Bradford, M. A. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.

Brandenburg, A. H.; Weller, C. L.; Testin, R. F. Edible films and coatings from soy protein. *J. Food Sci.* **1993**, *58*, 1086−1089.

Chatterji, A. K.; Arnold, L. K. Cross-linking of dialdehyde starches with wheat proteins. *J. Polym. Sci.: A* **1965**, *3*, 3857−3864.

Cheftel, J. C.; Cuq, J. L.; Lorient, D. Amino acids, peptides, and proteins. In *Food Chemistry*; Fennema, O. R., Ed.; Dekker: New York, 1985; pp 279, 289, 336, and 343.

Feeney, R. E.; Blankenhorn, G.; Dixon, H. B. F. Carbonyl-amine reactions in protein chemistry. *Adv. Protein Chem.* **1975**, *29*, 135−203.

Fukushima, D.; Van Buren, J. Mechanisms of protein insolubilization during the drying of soy milk. Role of disulfide and hydrophobic bonds. *Cereal Chem.* **1970**, *47*, 687−696.

Gennadios, A.; Weller, C. L. Edible films and coatings from soymilk and soy protein. *Cereal Foods World* **1991**, *36*, 1004−1009.

Gennadios, A.; Brandenburg, A. H.; Weller, C. L.; Testin, R. F. Effect of pH on properties of wheat gluten and soy protein isolate films. *J. Agric. Food Chem.* **1993**, *41*, 1835−1839.

Gennadios, A.; McHugh, T. H.; Weller, C. L.; Krochta, J. M. Edible coatings and films based on proteins. In *Edible Coatings and Films to Improve Food Quality*; Krochta, J. M., Baldwin, E. A., Nisperos-Carriedo, M., Eds.; Technomic Publishing: Lancaster, PA, 1994; pp 201−277.

Gennadios, A.; Ghorpade, V. M.; Weller, C. L.; Hanna, M. A. Heat curing of soy protein films. *Trans. ASAE* **1996**, *39*, 575−579.

Gennadios, A.; Rhim, J. W.; Handa, A.; Weller, C. L.; Hanna, M. A. Ultraviolet radiation affects physical and mechanical properties of soy protein films. *J. Food Sci.* **1998a**, *63*, 225−228.

Gennadios, A.; Handa, A.; Froning, G. W.; Weller, C. L.; Hanna, M. A. Physical properties of egg white-dialdehyde starch films. *J. Agric. Food Chem.* **1998b**, *46*, 1297−1302.

Ghorpade, V. M.; Li, H.; Gennadios, A.; Hanna, M. A. Chemically modified soy protein films. *Trans. ASAE* **1995**, *38*, 1805−1808.

Gontard, N.; Guilbert, S.; Cuq, J. L. Water and glycerol as plasticizers affect mechanical and water vapor barrier properties of an edible wheat gluten film. *J. Food Sci.* **1993**, *58*, 206−211.

Hakata, T.; Sato, H.; Watanabe, Y.; Matsumoto, M. Effect of formaldehyde on the physicochemical properties of soft gelatin capsule shells. *Chem. Pharm. Bull.* **1994**, *42*, 1138−1142.

Handa, A.; Gennadios, A.; Froning, G. W.; Kuroda, N.; Hanna, M. A. Tensile, solubility, and electrophoretic properties of egg white films as affected by surface sulfhydryl groups. *J. Food Sci.* **1999**, *64*, 82−85.

Harlan, J. W.; Feairheller, S. H. Chemistry of the cross-linking of collagen during tanning. In *Protein Cross-linking—Biochemical and Molecular Aspects*; Friedman, M., Ed.; Plenum Press: New York, 1977; pp 425−440.

Helmstetter, G. J. Gel strength enhancer for gelatin compositions including an oxidized polysaccharide. U.S. Patent 4,-055,554, 1977.

Hoshi, Y.; Yamauchi, F.; Shibasaki, K. On the role of disulfide bonds in polymerization of soybean 7S globulin during storage. *Agric. Biol. Chem.* **1982**, *46*, 2803−2807.

Jaynes, H. O.; Chu, W. N. New method to produce soy protein−lipid films. *Food Prod. Dev.* **1975**, *9* (4), 86, 90.

Krochta, J. M.; De Mulder-Johnston, C. Edible and biodegradable polymer films: Challenges and opportunities. *Food Technol.* **1997**, *51* (2), 61−74.

Krochta, J. M.; McHugh, T. H. Water-insoluble protein-based edible barrier coatings and films. U.S. Patent 5,543,164, 1996.

Kunte, L. A.; Gennadios, A.; Cuppett, S. L.; Hanna, M. A.; Weller, C. L. Cast films from soy protein isolates and fractions. *Cereal Chem.* **1997**, *74*, 115−118.

Lieberman, E. R.; Gilbert, S. G. Gas permeation of collagen films as affected by cross-linkage, moisture, and plasticizer content. *J. Polym. Sci.* **1973**, *41*, 33−43.

Marquié, C.; Aymard, C.; Cuq, J. L.; Guilbert, S. Biodegradable packaging made from cottonseed flour: Formation and improvements by chemical treatments with gossypol, formaldehyde, and glutaraldehyde. *J. Agric. Food Chem.* **1995**, *43*, 2762−2767.

Marquié, C.; Tessier, A. M.; Aymard, C.; Guilbert, S. HPLC determination of the reactive lysine content of cottonseed protein films to monitor the extent of cross-linking by formaldehyde, glutaraldehyde, and glyoxal. *J. Agric. Food Chem.* **1997**, *45*, 922−926.

McHugh, T. H.; Aujard, J. F.; Krochta, J. M. Plasticized whey protein edible films: Water vapor permeability properties. *J. Food Sci.* **1994**, *59*, 416−419, 423.

Mehltretter, C. L. Some landmarks in the chemical technology of carbohydrate oxidation. *Starch* **1963**, *15*, 313−319.

Miller, K. S.; Chiang, M. T.; Krochta, J. M. Heat curing of whey protein films. *J. Food Sci.* **1997**, *62*, 1189−1193.

Motoki, M.; Nio, N.; Takinami, K. Functional properties of heterologous polymer prepared by transglutaminase between milk casein and soybean globulin. *Agric. Biol. Chem.* **1987**, *51*, 237−239.

Nayudamma, Y.; Joseph, K. T.; Bose, S. M. Studies on the interaction of collagen with dialdehyde starch. *Am. Leather Chem. Assoc. J.* **1961**, *56*, 548−567.

Okamoto, S. Factors affecting protein film formation. *Cereal Foods World* **1978**, *23*, 256−262.

Parris, N.; Coffin, D. R.; Dickey, L. C.; Craig, J. C. Composition factors affecting the physical properties of hydrophilic zein films. In *Paradigm for Successful Utilization of Agricultural Resources*; Sessa, D. J., Willett, J. L., Eds.; AOCS Press: Champaign, IL, 1998; pp 255−265.

Rangavajhyala, N.; Ghorpade, V. M.; Hanna, M. A. Solubility and molecular properties of heat-cured soy protein films. *J. Agric. Food Chem.* **1997**, *45*, 4204−4208.

Rhim, J. W. Modification of soy protein film by formaldehyde. *Korean J. Food Sci. Technol.* **1998**, *30*, 372−378.

Rhim, J. W.; Gennadios, A.; Weller, C. L.; Cezeirat, C.; Hanna, M. A. Soy protein isolate-dialdehyde starch films. *Industr. Crops Prod.* **1998**, *8*, 195−203.

Rhim, J. W.; Wu, Y.; Weller, C. L.; Schnepf, M. Physical characteristics of a composite film of soy protein isolate and propyleneglycol alginate. *J. Food Sci.* **1999a**, *64*, 149−152.

Rhim, J. W.; Gennadios, A.; Fu, D.; Weller, C. L.; Hanna, M. A. Properties of ultraviolet irradiated protein films. *Lebensm. Wiss. Technol.* **1999b**, *32*, 129−133.

Rubin, A. L.; Riggio, R. R.; Nachman, R. L.; Schwartz, G. H.; Miyata, T.; Stenzel, K. H. Collagen materials in dialysis and implantation. *Trans. Am. Soc. Artif. Intern. Org.* **1968**, *14*, 169−175.

Sian, N. K.; Ishak, S. Effect of pH on yield, chemical composition, and boiling resistance of soybean protein−lipid film. *Cereal Foods World* **1990**, *35*, 748, 750, and 752.

Spence, K. E.; Jane, J. L.; Pometto, A. L., III. Dialdehyde starch and zein plastic: Mechanical properties and biodegradability. *J. Environ. Polym. Degrad.* **1995**, *3*, 69−74.

Stuchell, Y. M.; Krochta, J. M. Enzymatic treatments and thermal effects on edible soy protein films. *J. Food Sci.* **1994**, *59*, 1332−1337.

Tomihata, K.; Burczak, K.; Shiraki, K.; Ikada, Y. Cross-linking and biodegradation of native and denatured collagen. *Polym. Prepr.* **1992**, *33*, 534−535.

Weadock, K.; Olson, R. M.; Siver, F. H. Evaluation of collagen cross-linking techniques. *Biomater. Med. Dev. Art. Org.* **1984**, *11*, 293−318.

Weakley, F. P.; Ashby, M. L.; Mehltretter, C. L. Casein-dialdehyde starch adhesive for wood. *For. Prod. J.* **1963**, *13* (2), 51−55.

Welz, M. M.; Ofner, C. M., III. Examination of self-cross-linked gelatin as a hydrogel for controlled release. *J. Pharm. Sci.* **1992**, *81*, 85−90.

Were, L.; Hettiarachchy, N. S.; Coleman, M. Properties of cysteine-added soy protein-wheat gluten films. *J. Food Sci.* **1999**, *64*, 514−518.

Yamagishi, T.; Miyakawa, A.; Noda, N.; Yamauchi, F. Isolation and electrophoretic analysis of heat induced products of mixed soybean 7S and 11S globulins. *Agric. Biol. Chem.* **1983**, *47*, 1229−1237.

Yildirim, M.; Hettiarachchy, N. S. Biopolymers produced by cross-linking soybean 11S globulin with whey proteins using transglutaminase. *J. Food Sci.* **1997**, *62*, 270−275.

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