Physical Properties of Egg White−Dialdehyde Starch Films†

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Films were cast from heated (40 °C for 20 min) alkaline (pH 11.25) aqueous solutions of egg white (EW) solids (9 g/100 mL of water), polyethylene glycol 400 (60% w/w of EW), yolk solids (10% w/w of EW), and dialdehyde starch (DAS) at 0, 2.5, 5, 7.5, or 10% (w/w) of EW. For all types of films, tensile strength (TS), percentage elongation at break (*E*), Hunter color values (*L*, *a*, and *b*), total soluble matter (TSM) after immersion in water at 25 °C for 24 h, and protein solubility (PS) after immersion for 12 h in buffers (pH 8) containing urea and urea/2-mercaptoethanol were determined. DAS addition increased ($P < 0.05$) film TS and yellowness (+*b* values) and reduced ($P < 0.05$) film TSM and PS in both buffer systems. These modifications in film properties suggested occurrence of cross-linking between EW protein and DAS. This was further supported by SDS−PAGE patterns. Such patterns for DAS-containing films revealed bands of aggregates, increasing in intensity with increasing amounts of DAS, which were absent from patterns of control EW films.

Keywords: *Egg albumen; protein films; dialdehyde starch; cross-linking*

INTRODUCTION

There is increased interest in developing renewable, degradable, and compostible films and coatings from polysaccharide, protein, and lipid biopolymers for edible and nonedible packaging applications (Conca and Yang, 1993; Gontard and Guilbert, 1994; Krochta and De Mulder-Johnston, 1997). Research findings on protein-based films have been reviewed by Gennadios et al. (1994) and Torres (1994). Egg white (albumen) is a complex protein system consisting of ovomucin fibers in an aqueous solution of numerous globular proteins (Powrie and Nakai, 1986). In commercial production of dried egg white, liquid egg white is fermented to remove glucose and then spray-dried or pan-dried (Bergquist, 1986). The film-forming ability of egg white has been explored. Hydrophobic organic compounds used as food or cosmetic ingredients have been encapsulated in egg albumen (Soloway, 1964; Kosar and Atkins, 1968). Application of protective albumen coatings on raisins (Bolin, 1976) and shell eggs (Wong et al., 1996) has been investigated. Cast albumen films with added lysozyme inhibited bacterial growth, showing potential as active packagings (Padgett et al., 1995). Okamoto (1978) reported film formation on the surface of heated alkaline egg white solutions similar to formation of soy protein−lipid (yuba) films on the surface of heated soy milk. Recently, the mechanical and water vapor barrier properties of cast albumen films plasticized with glycerin, sorbitol, or polyethylene glycol have been determined (Gennadios et al., 1996a).

Functional properties of protein films can be improved by cross-linking through physical, chemical, or enzymatic treatments. Aldehydes such as formaldehyde, glutaraldehyde, and glyoxal promote inter- and intramolecular cross-linking in proteins (Habeeb and Hiramoto, 1968; Richards and Knowles, 1968; Feeney et al., 1975). The ϵ-amino group of lysine, the guanidino group of arginine, the imidazole ring of histidine, and the phenolic ring of tyrosine have shown ability to react with aldehydes (Habeeb and Hiramoto, 1968). In particular, the ϵ-amino group of lysine was considered the primary reactive site between proteins and aldehydes (Nayudamma et al., 1961; Quiocho and Richards, 1966). Formaldehyde, glutaraldehyde, or glyoxal has been used to cross-link films from collagen (Lieberman and Gilbert, 1973; Weadock et al., 1984; Tomihata et al., 1992), gelatin (Tomihata et al., 1992), corn zein (Clark and Gralow, 1949), soy protein (Ghorpade et al., 1995), and cottonseed proteins (Marquié et al., 1995, 1997). However, the inherent toxicity of the aforementioned aldehydes (Ernst et al., 1962; Speer et al., 1980) limits their functionality in improving properties of protein films and coatings.

Dialdehyde starch (DAS), a polymeric aldehyde (molecular weight range of 300 000−5 000 000) obtained by reacting native starch with periodic acid (Pfeifer et al., 1960; Mehltretter, 1963) has shown low toxicity to rats by oral, dermal, and respiratory routes of introduction (Wilson, 1959). Cross-linking of collagen (Nayudamma et al., 1961), casein (Weakley et al., 1961, 1963; Ernst

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et al., 1962), wheat gluten (Chatterji and Arnold, 1965), and corn zein (Spence at al., 1995) with DAS has been documented. Therefore, use of DAS for modifying properties of protein films and coatings merits investigation. Our objective was to study the effect of DAS on selected functional properties and on electrophoretic characteristics of cast egg white (EW) films.

MATERIALS AND METHODS

Film Preparation. Film-forming solutions were prepared by slowly dissolving spray-dried EW (desugared; minimum 92% solids; minimum 80% protein; Type P-39, Henningsen Foods, Inc., Omaha, NE) under constant stirring in distilled water (9 g/100 mL of water). To prevent foaming of EW, spray-dried egg yolk solids (minimum 95% solids; desugared; no additives; Type Y-2, Henningsen Foods, Inc.) were stirred in the water prior to EW addition at a level of 10% (w/w) of EW. The detrimental effect of small amounts of egg yolk on EW foaming is well documented (Baldwin, 1986). Polyethylene glycol with average molecular weight of 400 (PEG 400; Fisher Scientific, Pittsburgh, PA), a plasticizer, was added at 60% (w/w) of EW. After the pH values of solutions were adjusted to 11.25 ± 0.02 with 2 N sodium hydroxide, DAS (81.8% starch oxidation, Sigma Chemical Co., St. Louis, MO) was slowly mixed in at 0, 2.5, 5, 7.5, or 10% (w/w) of EW. The pH of 11.25 was selected to obtain homogeneous, smooth films (Gennadios et al., 1996a). Solutions were heated in a water bath at 40 °C for 20 min, strained through cheesecloth (grade 40, Fisher Scientific), and cast on leveled Teflon-coated glass plates (21 cm × 35 cm). To control film thickness (130 ± 13 μm), the quantity of each film-forming solution poured onto a plate was calculated so that the solids content (EW, yolk solids, DAS, and PEG 400) was the same (70 mL for control film-forming solutions). Castings were kept at ambient conditions for 4−5 h to solidify and then placed in an environmental chamber at 25 °C and 50% relative humidity (RH). After ~15 h in the chamber, films were peeled from the plates and cut to specimens of appropriate sizes for subsequent tensile, color, total soluble matter, and protein solubility testing.

Tensile Testing. Film tensile strength (TS) and percentage elongation at break (*E*) were determined with an Instron universal testing machine (model 5566, Instron Corp., Canton, MA). Initial grip separation was set at 10 cm, and cross-head speed was set at 5 cm/min. Ten thickness measurements were taken along each specimen (2.5 cm × 15 cm) with a hand-held micrometer (B. C. Ames Co., Waltham, MA) to the nearest 2.54 μm (0.1 mil), and mean thickness was used in TS calculations. TS was calculated by dividing maximum load (force) by initial cross-sectional area of a specimen. *E* was expressed as percentage of change of initial gauge length of a specimen (10 cm) at the point of sample failure. Prior to tensile testing, film specimens were conditioned for 3 days in an environmental chamber at 25 °C and 50% RH according to ASTM Standard Method D 882-91 (ASTM, 1995). In a slight deviation from this ASTM method, tensile testing of film samples was conducted, as quickly as possible, in ambient conditions rather than in the recommended Standard Laboratory Atmosphere of 23 ± 2 °C and 50 ± 5% RH. TS and *E* values for each type of film were determined in quadruplicate with individually prepared and cast films as the replicated experimental units. Each TS and *E* replicate value was the mean of five or six tested sampling units (specimens) taken from the same film.

Color. Color values of films were measured with a portable colorimeter (CR-300 Minolta Chroma Meter, Minolta Camera Co., Ltd., Osaka, Japan). Film specimens (7 cm × 7 cm) were placed on a white standard plate (calibration plate CR-A43) and the HunterLab color scale was used to measure color: $L = 0$ (black) to $L = 100$ (white); $a = -80$ (greenness) to $a = 100$ (redness); and $b = -80$ (blueness) to $b = 70$ (yellowness). Five measurements were taken at different locations on each specimen, one at the center and four around the perimeter. Color measurements for each type of film were replicated four times with individually prepared films as the replicated experimental units and each replicate being the mean of two tested sampling units (specimens) taken from the same film.

Total Soluble Matter (TSM). TSM was expressed as percentage of film dry matter solubilized after 24 h of immersion in distilled water. Film specimens (2 cm × 2 cm) that had been stored in an environmental chamber at 25 °C and 50% RH for 3 days were weighed (±0.0001 g) and transferred into 50 mL beakers containing 30 mL of distilled water. Traces of sodium azide (0.1% w/v) also were added to inhibit microbial growth. The beakers were covered with Parafilm "M" wrap (American National Can, Greenwich, CT) and stored in an environmental chamber at 25 °C for 24 h with occasional gentle stirring. Undissolved dry matter was determined by removing the film pieces from the beakers and drying them in an air-circulating oven (105 °C for 24 h). The weight of solubilized dry matter was calculated by subtracting the weight of unsolubilized dry matter from the initial weight of dry matter. Initial dry matter and soluble matter were not determined on the same film specimens from each cast film to avoid heating film samples prior to incubation in water. Increases in water resistance as a result of heat-induced cross-linking have been reported for protein films from corn zein (Howland, 1961; Howland and Reiners, 1962; Julius, 1967), gelatin (Julius, 1967; Welz and Ofner, 1992), collagen (Lieberman, 1967; Weadock et al., 1984), and soy protein isolate (Gennadios et al., 1996b). Instead, the initial dry matter of each cast film required for TSM calculations was determined on different specimens (two from each film) by drying in an air-circulating oven (105 °C for 24 h). TSM for each type of film was determined in triplicate with individually prepared and cast films as the replicated experimental units. Each TSM replicate value was the mean of two tested sampling units (specimens) taken from the same film.

Protein Solubility. Soluble protein of films in urea and in urea/2-mercaptoethanol was determined. Film specimens (2 cm × 2 cm) that had been stored in an environmental chamber at 25 °C and 50% RH for 3 days were weighed (±0.0001 g) and transferred into glass test tubes with 10 mL of either 50 mM Tris-HCl buffer (pH 8)/4 M urea or 50 mM Tris-HCl buffer (pH 8)/4 M urea/0.2 M 2-mercaptoethanol. The tubes were fitted with screw caps and shaken for 12 h at room temperature (≈23 °C) using a reciprocating shaker (Eberbach Corp., Ann Arbor, MI). Following filtration with Whatman No. 4 filter paper (Whatman International Ltd., Maidstone, England), protein concentration in the solvent was measured using a Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA) as described by Bradford (1976). Ovalbumin (minimum 98% albumin, Sigma Chemical Co.) was used as the standard for protein quantitation. Similar to TSM measurements, film specimens were immersed in the solvents without prior oven-drying to determine their initial dry matter. Instead, the initial dry matter of each cast film after 3 days in 25 °C and 50% RH was determined on different specimens (two from each film) by drying in an air-circulating oven (105 °C for 24 h). Protein solubility (PS) was expressed as percentage of total protein in the film that was solubilized. Total protein in films was calculated from the compositions of film-forming solutions. Actual protein contents of EW solids (duplicate) and yolk solids (triplicate) were measured according to the Kjeldahl method (nitrogen factor = 6.25) using a block digester (AOAC, 1990). Mean protein content values (wet basis) of EW (80.52 ± 0.03%) and yolk solids (32.06 ± 0.30%) were used in calculating total protein content in films. PS in each of the two solvents for each type of film was determined in triplicate with individually prepared and cast films as the replicated experimental units. Each PS replicate value was the mean of two tested sampling units (specimens) taken from the same film.

Electrophoresis. Molecular characteristics of native EW solids, control EW films, and EW−DAS films were compared through electrophoretic patterns. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was performed according to the method of Laemmli (1970) with an Xcell II Mini-Cell unit (Novex, San Diego, CA). A 4% acrylamide stacking gel and a 4−20% gradient separating gel were

Table 1. TS, *E*, and *L*, *a*, and *b* Color Values of EW Films with Various Levels [Percent (w/w) of EW] of Added DAS[a]

DAS (%)	TS (MPa)	*E* (%)	*L*	*a*	*b*
0	4.65 ± 0.60^{b}	58.4 ± 18.1^{b}	95.36 ± 0.04^{f}	-1.20 ± 0.06^{f}	7.04 ± 0.17^{b}
2.5	5.60 ± 0.27^{c}	74.1 ± 16.7^{bc}	93.91 ± 0.24^{e}	-3.91 ± 0.06^{e}	19.0 ± 0.21^{c}
5	6.26 ± 0.35^{d}	78.6 ± 29.0^{c}	92.72 ± 0.15^{d}	-4.99 ± 0.10^{b}	27.14 ± 0.71^{d}
7.5	6.64 ± 0.48^{d}	75.2 ± 11.2^{bc}	91.80 ± 0.10^{c}	-4.82 ± 0.09^{c}	30.44 ± 0.60^{e}
10	6.55 ± 0.48^{d}	67.6 ± 9.7^{bc}	91.54 ± 0.08^{b}	-4.40 ± 0.06^{d}	31.08 ± 0.62^{e}

[a] Means of four 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.

used. Samples (≈1 mg/mL) were dissolved in 0.0625 M Tris-HCl buffer (pH 6.8) containing SDS (2% w/v), glycerol (7% v/v), bromophenol blue (0.005% w/v), urea (8 M), and 2-mercaptoethanol (5% w/v). Electrophoresis was carried out at the constant voltage of 125 V/slab. Gels were stained with Coomassie brilliant blue R-250 (0.1% w/v) in methanol/acetic acid/water (40/10/50% v/v/v) and were destained in methanol/acetic acid/water (10/7.5/82.5% v/v/v). The standard protein mixture (Novex Mark 12, San Diego, CA) consisted of myosin (200.0 kDa), β-galactosidase (116.3 kDa), phosphorylase B (97.2 kDa), bovine serum albumin (66.3 kDa), glutamic dehydrogenase (55.4 kDa), lactate dehydrogenase (36.5 kDa), carbonic anhydrase (31.0 kDa), trypsin inhibitor (21.5 kDa), lysozyme (14.4 kDa), apotinin (6.0 kDa), insulin B chain (3.5 kDa), and insulin A chain (2.5 kDa).

Statistical Analysis. Statistical analysis on a completely randomized experimental design was performed using the General Linear Models procedure in SAS (release 6.08, SAS Institute, Inc., Cary, NC) software (Littell et al., 1991). Significantly ($P < 0.05$) different property means were separated with Duncan's multiple range test.

RESULTS AND DISCUSSION

TS and Elongation. TS of EW films increased ($P < 0.05$) with addition of DAS (Table 1), suggesting occurrence of cross-linking between EW and DAS. Substantial amounts of lysine (51.2 mg/g), arginine (46.5 mg/g), tyrosine (31.8 mg/g), and histidine (18.6 mg/g) are present in dried EW (Cook and Briggs, 1986). As mentioned, these amino acids are believed to react with aldehydes. Similar to the present study, DAS addition improved mechanical strength of gelatin films (Helmstetter, 1977). Molded DAS/zein (3:1) bioplastics had twice the TS of native starch/zein (3:1) bioplastics due to DAS-induced cross-linking of zein (Spence et al., 1995). Furthermore, increases in TS and/or puncture strength have been reported for formaldehyde-treated collagen/gelatin casings (Lieberman, 1967); glutaraldehyde-, acetaldehyde-, valeraldehyde-, and benzaldehyde-treated wool keratin fabrics (Di Monica and Marzona, 1971); glutaraldehyde-treated collagen films (Weadock et al., 1984); formaldehyde-treated soy protein isolate films (Ghorpade et al., 1995); cottonseed protein films reacted with formaldehyde, glutaraldehyde, glyoxal, and gossypol (Marquié et al., 1995, 1997); and formaldehyde- and glutaraldehyde-treated molded bioplastics from starch/zein, zein, soy protein isolate, and wheat gluten (Jane et al., 1993; Lim and Jane, 1993). EW film TS increased by approximately 20 and 35% when DAS was incorporated at 2.5 and 5%, respectively. Further increasing the amount of added DAS from 5 to 7.5 or 10% did not affect significantly ($P > 0.05$) film TS (Table 1). This probably indicated that the cross-linking reaction between EW protein and DAS reached a "saturation" point at a DAS level ∼5%. Although additional reactive groups might have been available along protein chains, steric hindrances most likely prevented aldehydic functions on the polymeric DAS from interacting with remaining reactive amino acid groups.

Increased TS of protein films due to cross-linking often is accompanied by reduced film *E* (less extensible films) as the cross-links result in a more rigid film structure. For example, greater TS and lower *E* compared to control films have been reported for collagen films cross-linked by glutaraldehyde, heat curing, or ultraviolet radiation (Weadock et al., 1984) and for soy protein isolate films cross-linked by heat curing (Gennadios et al., 1996b). Control EW films in the present study had a slightly lower *E* than films with DAS (Table 1). However, differences in *E* between control and DAS-containing films were mainly insignificant ($P > 0.05$). Therefore, in contrast to cross-linking by physical treatments and by low molecular weight chemical agents, DAS did not cause increased rigidity and "tightness" of film matrixes. Apparently, since DAS is itself a biopolymer, the macromolecular DAS chains "fixed" within the protein film structure did not restrict the flexibility of the protein matrix.

Color. Addition of DAS to EW films caused yellowness as indicated by a substantial increase ($P < 0.05$) in $+b$ values (Table 1). Moreover, DAS-containing films had slightly lower ($P < 0.05$) *L* (decreased lightness) and $-a$ (increased greenness) values. In general, interactions between proteins and bifunctional aldehydes are associated with generation of yellow/brownish coloration due to various intermediates or final products of the Maillard reaction (Cheftel et al., 1985). Therefore, the yellowness of DAS-containing EW films suggested development of cross-linking within the film structure. Film yellowness increased drastically as the amount of DAS in films increased from 2.5 to 5% (greater $+b$ mean value by ∼43%). However, further increasing the DAS amount to 7.5 or 10% had a slight effect on film yellowness ($+b$ values). This suggested that, in agreement with TS data, the cross-linking reaction between EW protein and DAS approached a "saturation" point at a DAS concentration of ∼5% (w/w) of EW. Reportedly, DAS produced a yellow to brown color when reacted with collagen in neutral or alkaline pH (Nayudamma et al., 1961) and with casein in alkaline conditions (Weakley et al., 1961). From the infrared spectrum of the brown compound formed in the reaction of DAS with amino acids, Nayudamma et al. (1961) concluded that the compound had a Schiff base structure. Molded DAS−zein (3:1) bioplastics were darker than native starch−zein (3:1) bioplastics (Spence et al., 1995). Also, low molecular weight aldehydes, such as glutaraldehyde and glyceraldehyde, produced yellow coloration when reacted with various proteins (Happich et al., 1965; Wold, 1967; Habeeb and Hiramoto, 1968; Jones and Whitmore, 1972).

TSM. In general, protein films have low water resistance due to the inherent hydrophilicity of proteins and the large amounts of added hygroscopic plasticizers (Gennadios et al., 1994). Therefore, cross-linking treatments capable of improving moisture resistance of

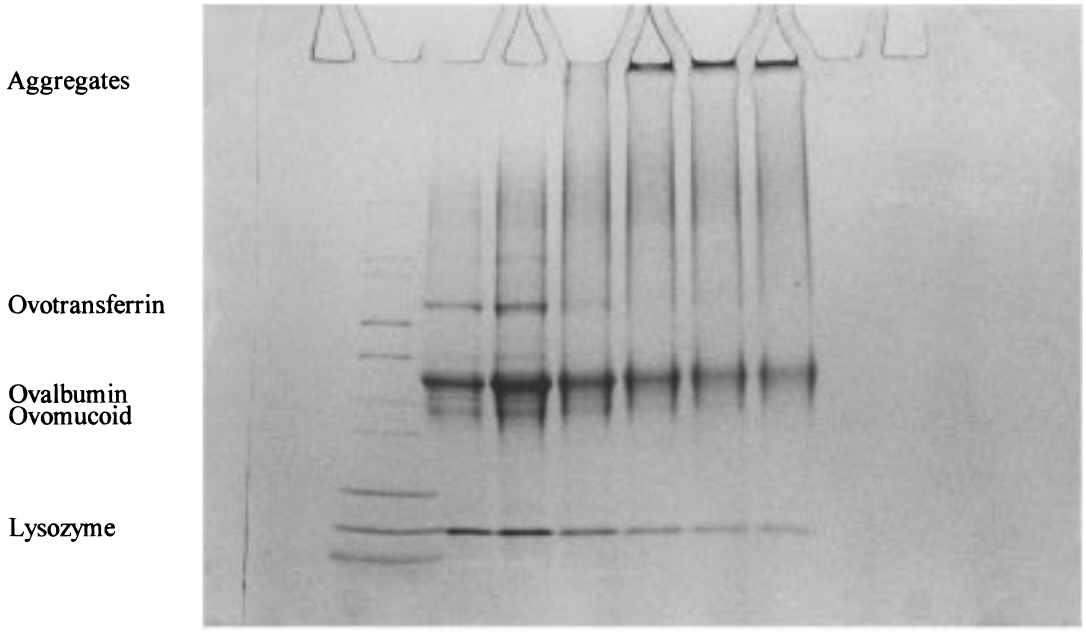

Figure 1. SDS−PAGE patterns for native EW solids (lane 2) and for cast films from EW and DAS at 0, 2.5, 5, 7.5, and 10% (w/w) of EW (lanes 3−7, respectively). Lane 1 is molecular weight standard.

Table 2. TSM (Percent Dry Basis) in Water, PS (Percent Dry Basis) in Urea (UPS), and PS (Percent Dry Basis) in Urea/2-Mercaptoethanol (UMPS) of EW Films with Various Levels (Percent w/w of EW) of Added DAS[a]

DAS (%)	TSM	UPS	UMPS
0	52.2 ± 2.1^{b}	45.5 ± 3.6^{b}	64.3 ± 4.7^{b}
2.5	44.7 ± 0.3^{c}	24.6 ± 3.6^{c}	51.6 ± 4.1^{c}
5	43.3 ± 0.1^{cd}	20.1 ± 1.4^{d}	37.5 ± 2.1^{d}
7.5	41.8 ± 0.5^{d}	14.4 ± 1.3^{e}	25.9 ± 4.2^{e}
10	41.7 ± 0.7^{d}	11.3 ± 0.9^{e}	21.8 ± 3.2^{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.

protein films are desirable. TSM of EW films decreased significantly ($P < 0.05$), by ~15%, when DAS was added at 2.5 or 5% (Table 2). No further reduction in film TSM was noticed by increasing incorporated DAS to 7.5 or 10% (Table 2). The reduced solubility in water offered additional indirect evidence that DAS-induced cross-linking of EW protein occurred within films. As mentioned, sites on protein chains susceptible to cross-linking by aldehydes include the amino, guanidino, imidazole, and phenolic groups. These groups associate with water through hydrogen bonding. As these hydrophilic groups were fixed by DAS, EW films absorbed less water and became less soluble. Furthermore, increased protein molecular weight due to DAS-induced cross-linking could also have contributed to the solubility increase. Reportedly, DAS irreversibly insolubilized casein (Weakley et al., 1961). DAS−zein (3:1) bioplastics exhibited substantially lower water absorption than native starch−zein (3:1) bioplastics (Spence et al., 1995). Low molecular weight aldehydes also have been effectively used for improving water resistance of protein films. For example, formaldehyde imparted greater water resistance to zein films (Clark and Gralow, 1949); glyceraldehyde inhibited water deformation of collagen and gelatin films (Jones and Whitmore, 1972); formaldehyde reduced TSM of soy protein isolate films (Ghorpade et al., 1995); and formaldehyde and glutaraldehyde reduced TSM of cottonseed protein films (Marquié et al., 1995). Loss of solubility in gelatin capsules due to cross-linking by formaldehyde or other aldehydes is well documented (Digenis et al., 1994; Hakata et al., 1994). Also, water absorption by molded bioplastics from zein, wheat gluten, or soy protein isolate decreased substantially after cross-linking with formaldehyde (Jane et al., 1993).

PS. PS values of control EW and EW−DAS films in urea and in urea/2-mercaptoethanol are shown in Table 2. It is believed that covalent disulfide bonds play an important role in film formation by various plant and animal proteins and that weaker hydrophobic and hydrogen bonds also are involved (Wall and Beckwith, 1969; Okamoto, 1978; Gennadios et al., 1994; McHugh and Krochta, 1994). Most likely, similar interactions occur in EW film formation since involvement of disulfide and hydrophobic bonds in gelation of egg albumen has been demonstrated (Shimada and Matsushita, 1980; Ma and Holme, 1982; Hayakawa and Nakai, 1985; Margoshes, 1990; Mine et al., 1990). Urea disrupts hydrophobic and hydrogen bonds, whereas 2-mercaptoethanol breaks (reduces) disulfide bonds (Cheftel et al., 1985). Addition of DAS significantly ($P < 0.05$) reduced PS in both urea and urea/2-mercaptoethanol (Table 2). PS of EW films with 10% DAS in urea and in urea/2-mercaptoethanol was lower by 75 and 65%, respectively, compared to control EW films. This showed that bonds other than disulfide, hydrophobic, or hydrogen bonds were present in DAS-containing EW films, thus further suggesting occurrence of DAS-induced cross-linking. PS in both solvents was lower for films with 10% DAS than for films with 7.5% DAS. However, this difference was not significant ($P > 0.05$), indicating that development of protein−DAS cross-links reached a maximum at a DAS level between 5 and 7.5%. For all types of films, PS in both solvents was substantially greater in urea/2-mercaptoethanol than in urea (Table 2). This offered evidence that disulfide bonds, which were cleaved by 2-mercaptoethanol, played a major role in the EW film structure.

Electrophoresis. SDS−PAGE patterns of native EW solids, control EW films, and EW−DAS films are shown in Figure 1. Addition of DAS reduced the intensity of the bottom bands corresponding to highly mobile protein fractions. At the same time, aggregate bands, increasing in intensity with increasing amounts of DAS, were evident in EW−DAS films. The buffer system used for preparing film samples for electrophoresis included disulfide bond-cleaving 2-mercaptoethanol. Therefore, the observed aggregates in electrophoretic patterns of EW−DAS films were attributed to covalent cross-linking between DAS and protein rather than to intermolecular disulfide bonds between protein chains.

Implications. This study showed that mechanical strength and water solubility of cast EW films can be increased with addition of DAS at a level of 5−7.5% w/w of EW. These modifications in functional properties may improve performance of such films in renewable packaging applications. The fact that DAS is itself a biopolymer makes its use as a component of biodegradable films even more appealing. Although more expensive than low molecular weight aldehydes, DAS presents a nontoxic alternative for cross-linking protein films and coatings. Use of DAS for this purpose will create a niche outlet for a value-added, modified starch product.

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