# Effect of pH on Properties of Wheat Gluten and Soy Protein Isolate Films[†]

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Soy protein isolate (SPI) and wheat gluten (WG) films can be cast from appropriate solvent systems. Such films can find use as edible or nonedible food packaging materials. The effect of pH of WG and SPI film-forming solutions on film formation, tensile strength (TS), percentage elongation at break ($E$), and water vapor permeability (WVP) was investigated. WG films formed within pH 2–4 and 9–13, whereas SPI films formed within pH 1–3 and 6–12. Film formation was inhibited by poor protein dispersion around the isoelectric pH region of SPI (pH 4.5) and WG (pH 7.6). SPI films prepared from pH 6 to 11 had significantly ($P < 0.05$) higher TS, higher $E$, and lower WVP than films from pH 1 to 3. WG films produced under alkaline conditions had significantly ($P < 0.05$) higher TS than films processed under acidic conditions.

## INTRODUCTION

Edible films and coatings produced from renewable biological substances hold promise for innovative uses as food protective materials. Placed on the outer surface or in the interior of food systems, edible films could function as selective barriers to movement of gases, vapors, and solutes. The concept has found some commercial application in the form of collagen and cellulose sausage casings (Rust, 1987; Hood, 1987), gelatin capsules for medication and nutritional supplements (Viro, 1980), corn zein-based coatings for confectionery products (Alikonis, 1979; Andres, 1984); and fresh produce coatings from waxes (Trout et al., 1953; Lawrence et al., 1982; Lawrence and Iyengar, 1983; Kaplan, 1986) and sucrose fatty acid polyesters (Lowings and Cutts, 1982; Elson et al., 1985; Santerre et al., 1989; Nisperos-Carriedo et al., 1990). Interest in edible packaging is likely to continue to grow based on food processor and consumer needs for foods of increased quality and longer shelf life, opportunities for further utilization of agricultural commodities employed in production of edible films, and potential for reducing use of food packaging materials from nonrenewable sources.

Recent reviews have compiled information on film formation from several polysaccharide, lipid, and protein compounds (Kester and Fennema, 1986; Guilbert, 1986, 1988; Krochta, 1992). Proteins have received less study as film formers than lipids and polysaccharides. Soy protein isolate (SPI) and wheat gluten (WG) are among the proteins that have been investigated for film development. Research findings related to formation, properties, and potential applications of WG and SPI films have recently been reviewed by Gennadios and Weller (1990, 1991).

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Edible soy protein–lipid films have been traditionally produced in the Orient on the surface of heated soymilk (Wang, 1981). Films successively form on the soymilk surface until the solution gels. Simple devices such as L-shaped wires and glass or stainless steel rods are employed for film removal (Gennadios and Weller, 1991). Films prepared in this manner (heat-catalyzed protein polymerization through surface dehydration) have been studied (Wu and Bates, 1972a,b, 1973, 1975; Watanabe et al., 1975; Farnum et al., 1976; Chuah et al., 1983; Sian and Ishak, 1990a,b). Films have also been prepared on the surface of heated SPI (Wu and Bates, 1972a, 1973, 1975; Okamoto, 1978) and WG (Okamoto, 1978) solutions containing no lipids or carbohydrates.

Another method of manufacturing protein films, which holds more interest for film production on a commercial scale, is deposition of protein dispersions in appropriate solvent systems. A few recent studies reported findings on development and property evaluation of WG (Gennadios et al., 1990, 1992, 1993a; Park and Chinnan, 1990; Aydt et al., 1991; Gennadios and Weller, 1992; Gontard et al., 1992) and SPI (Guilbert, 1986, 1988; Brandenburg et al., 1993) films produced by drying cast protein solutions. Various film-forming variables, such as the pH of film-forming solutions, need to be examined to determine their effect on protein film properties. The present study was undertaken to determine the effect of the pH of film-forming solutions on tensile strength, percentage elongation at break, and water vapor permeability of WG and SPI films.

## MATERIALS AND METHODS

**Reagents.** Vital wheat gluten (Do-Pep) with a protein content of 75% (dry basis) was donated by ADM Arkady, Olathe, KS. Soy protein isolate (PP 620) with a protein content of 91.5% (dry basis) was purchased from Protein Technologies International, St. Louis, MO. Glycerin (ACS grade), which was added to films as a plasticizer, was purchased from Fisher Scientific, Pittsburgh, PA.

**Preparation of Film-Forming Solutions.** Soy protein isolate (SPI) film-forming solutions of 5% (w/v) SPI and 3% (w/v) glycerin in distilled water were prepared. For wheat gluten (WG) films, mixtures comprised of 12.0% WG, 4.4% glycerin, 45.4% ethanol (95%), and 38.2%

distilled water on a wet weight basis were prepared. To obtain free-standing films, it was necessary to use higher glycerin concentration with SPI solutions (60% w/w of protein) than with WG solutions (37% w/w of protein). The pH of the solutions was appropriately adjusted with hydrochloric acid or sodium hydroxide. Subsequently, solutions were heated and stirred on a magnetic stirrer/ hot plate for 20 (SPI solutions) or 10 min (WG solutions). Heating rate was adjusted such that both SPI and WG solutions had a temperature of 75–80 °C at the end of the heating period. Upon removal from the hot plate, mixtures were kept at room conditions for 2–3 min to allow bubbling to cease prior to casting.

**Casting and Drying.** Solutions were poured and spread onto Teflon-coated glass plates leveled to the nearest 0.01 mm (master precision level, No. 199, The L. S. Starrett Co., Athol, MA). Solutions were poured and dried to produce SPI and WG films with mean thicknesses of 83 ± 7 and 150 ± 11 $\mu$m, respectively. Different thicknesses of WG and SPI films resulted from inherent variabilities between the two proteins and from use of different film-forming solutions in terms of solvent (water vs ethanol/water), protein concentration, and plasticizer concentration. Cast SPI solutions were allowed to dry for about 15 h at ambient temperature ($\approx$23 °C). Plates with WG solutions were kept at ambient temperature for 5–10 min and then placed for about 12 h in an air-circulating oven set at 43 °C. After drying, films were peeled from plates and specimens for property testing were cut. Tensile testing specimens were 100 mm × 25.4 mm rectangular strips. Water vapor transmission testing specimens were 70 mm × 70 mm in size.

**Thickness Measurement.** Film thickness was measured to the nearest 2.54 $\mu$m (0.1 mil) 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 strip with the mean used in tensile strength calculations. Similarly, five measurements were taken on each water vapor transmission specimen, one at the center and four around the perimeter, and the mean was used in water vapor permeability calculations.

**Conditioning.** Prior to testing, all film specimens were conditioned for 2 days in an environmental chamber (Model 317332, Hotpack, Corp., Philadelphia, PA) set at 50% relative humidity and 25 °C.

**Tensile Testing.** Tensile testing for determining tensile strength (TS) and percentage elongation at break (E) was performed with an Instron universal testing instrument (Model 4201, Instron Engineering Corp., Canton, MA). Initial grip separation was set at 50 mm, and cross-head speed was set at 500 mm/min. Calculations were performed as described by Gennadios et al. (1990).

**Water Vapor Permeability.** Water vapor permeability (WVP) or water vapor permeability constant was calculated using (Karel et al., 1959)

$$WVP = WVTR(L/\Delta p) \qquad (1)$$

where WVTR was the water vapor transmission rate of films measured at 25 °C and 50% relative humidity gradient, $L$ was the mean thickness of film specimens, and $\Delta p$ was the difference in partial water vapor pressure between the two sides of film specimens ($\approx$1583 Pa).

WVTR was determined gravimetrically using a variation of the ASTM E 96 Standard Method (ASTM, 1989), known as the cup method. Cups used for testing consisted of a cylindrical bottom (8.7-cm diameter) made of poly(methyl methacrylate) (Piedmont Plastics, Inc., Greenville, SC),

a lid of the same material, and a rubber O-ring. A well (4.6-cm diameter; 2.1-cm depth) was milled into the bottom. An O-ring (5.6-cm i.d.) was placed into a groove milled around the well. Four screws, symmetrically placed around the cup perimeter, were tightened to securely hold film specimens between the lid and the bottom. Good sealing of film specimens between lid and bottom was provided by the O-rings. The wells in the cups were filled with distilled water (100% relative humidity). After film specimens were mounted, the whole assembly was weighed and placed in an environmental chamber (Model 317332, Hotpack) set at 25 °C and 50% relative humidity. Subsequent weighings of cups to the nearest 0.001 g were taken at approximately 1-h intervals for a period of 12 h. Weight loss was plotted vs time, and a straight line (steady state) was obtained after about 2 h. The slope of the straight line was calculated with linear regression. WVTR was estimated by dividing this slope with the open mouth area of the cup (16.6 cm$^2$). WVTR values were corrected to account for resistance of the stagnant air gap (1 cm) between the underside of film samples mounted on cups and the surface of water inside cups (Krochta, 1992; Gennadios et al., 1993b).

**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 film property mean values at different pH values. TS, $E$, and WVP for each type of film at various pH values were determined in triplicate with individually prepared and cast 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, while each WVP replicate was the mean of three sampling units taken from the same film.

## RESULTS AND DISCUSSION

**SPI Film Formation.** Formation of homogeneous, free-standing SPI films was achieved within pH 1–3 and 6–12. No film formation occurred between pH 4 and 5. This pH region encloses the isoelectric point (pH 4.5) of soy protein (Cheftel et al., 1985). SPI coagulated, rather than dispersed, at pH 4–5, thereby not allowing for casting of mixtures. A similar observation, lack of film formation at the protein's isoelectric region, was reported for soy protein–lipid films formed on the surface of heated soymilk (Sian and Ishak, 1990b). Intermolecular covalent disulfide linkages and, secondarily, hydrophobic and hydrogen interactions are believed to be the main associative forces involved in soy protein film formation (Farnum et al., 1976; Okamoto, 1978; Sian and Ishak, 1990b). At pH values away from the isoelectric region, protein denatures, unfolds, and solubilizes, exposing sulfhydryl and hydrophobic groups. Such groups associate upon drying to form disulfide and hydrophobic bonding forces. However, extreme acidic (pH <1) and alkaline conditions (pH >12) inhibited SPI film formation. Most likely, strong repulsive forces between highly negative (extreme alkaline pH) or positive (extreme acidic pH) charges along protein chains prevent protein molecules from associating and forming films.

**WG Film Formation.** A pH range of 10–11 was recommended for WG film formation on the surface of heated WG solutions, in a manner similar to soy protein–lipid film formation on the surface of heated soymilk (Okamoto, 1978). Homogeneous, free-standing WG films were formed within broader pH ranges (pH 2–4 and 9–13) in the present study by deposition of protein solutions.

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*J. Agric. Food Chem.*, Vol. 41, No. 11, 1993 **1837**

**Table I. Tensile Strength (TS), Percentage Elongation at Break (*E*), and Water Vapor Permeability (WVP) of Soy Protein Isolate Films Cast from Solutions of Different pH[a]**

| pH | TS, MPa | *E*, % | WVP[b] × 10⁹, g/(m s Pa) |
|---|---|---|---|
| 1 | 1.9 ± 0.9 cd | 34.2 ± 11.8 e | 5.4 ± 1.5 a |
| 2 | 2.3 ± 0.3 bc | 49.3 ± 8.9 de | 4.0 ± 0.4 bc |
| 3 | 1.9 ± 0.6 cd | 35.6 ± 19.1 e | 5.0 ± 0.6 ab |
| 6 | 3.5 ± 0.2 a | 72.2 ± 13.2 d | 3.6 ± 0.2 c |
| 7 | 3.0 ± 0.4 ab | 132.6 ± 16.8 c | 3.2 ± 0.7 c |
| 8 | 3.6 ± 0.4 a | 139.5 ± 19.5 bc | 3.3 ± 0.2 c |
| 9 | 3.6 ± 0.3 a | 160.3 ± 29.5 abc | 3.5 ± 0.2 c |
| 10 | 3.6 ± 0.1 a | 169.3 ± 9.3 ab | 3.2 ± 0.4 c |
| 11 | 3.1 ± 0.1 ab | 187.3 ± 12.5 a | 2.9 ± 0.2 c |
| 12 | 1.3 ± 0.5 d | 66.5 ± 31.6 de | 2.9 ± 0.4 c |

[a] Films were 83 ± 7 μm thick. Reported values for each property are means of three replicates plus/minus one standard deviation. Any means followed by the same small-case letter are not different (*P* < 0.05) according to Duncan's multiple-range test. [b] Determined at 50% relative humidity gradient and 25 °C.

**Table II. Tensile Strength (TS), Percentage Elongation at Break (*E*), and Water Vapor Permeability (WVP) of Wheat Gluten Films Cast from Solutions of Different pH[a]**

| pH | TS, MPa | *E*, % | WVP[b] × 10⁹, g/(m s Pa) |
|---|---|---|---|
| 2 | 0.7 ± 0.2 e | 229.2 ± 83.7 | 3.4 ± 0.1 bc |
| 3 | 0.9 ± 0.3 e | 259.6 ± 46.0 | 3.4 ± 0.4 bc |
| 4 | 0.5 ± 0.2 e | 156.7 ± 61.6 | 4.7 ± 0.2 a |
| 9 | 1.9 ± 0.2 d | 208.0 ± 13.7 | 4.3 ± 0.6 a |
| 10 | 2.7 ± 0.3 c | 197.0 ± 31.2 | 4.3 ± 0.2 a |
| 11 | 3.3 ± 0.2 b | 191.9 ± 12.0 | 4.4 ± 0.4 a |
| 12 | 4.4 ± 0.7 a | 194.7 ± 28.3 | 4.0 ± 0.2 ab |
| 13 | 4.4 ± 0.5 a | 170.0 ± 58.7 | 3.2 ± 0.7 c |

[a] Films were 150 ± 11 μm thick. Reported values for each property are means of three replicates plus/minus one standard deviation. Any means followed by the same small-case letter are not different (*P* < 0.05) according to Duncan's multiple-range test. No differences (*P* < 0.05) were detected among *E* values. [b] Determined at 50% relative humidity gradient and 25 °C.

The isoelectric points of gliadin and glutenin, the two main protein fractions in WG, were estimated at 8.1 and 7.1, respectively (Wu and Dimler, 1963a), while the isoelectric point of the whole WG was estimated at 7.5 (Wu and Dimler, 1963b). Similarly to SPI, WG films did not form in the protein's isoelectric region (pH 7–8). Also, WG dispersion at pH 5–6 was very poor, resulting in films of uneven thickness and containing big particles of coagulated protein particles, that were unsuitable for property evaluation. Film formation was most likely impeded by intermolecular protein repulsive forces at extreme acidic (pH <2) and alkaline (pH >13) conditions.

**Tensile Strength (TS).** TS is an important mechanical property that expresses the maximum stress developed in a film during tensile testing (Briston, 1988). SPI films of higher TS were produced at pH values above the protein's isoelectric point (Table I). However, at high alkaline conditions (pH 12), TS was significantly (*P* < 0.05) reduced. On the average, SPI films from pH within 6–11 had higher (*P* < 0.05) TS by about 1.4 ± 0.4 MPa than films prepared from pH within 1–3. TS did not change significantly (*P* < 0.05) within pH 6–11.

Similar to SPI, WG films of higher TS were produced at pH values (pH 9–13) above the protein's isoelectric region (Table II). On the average, WG films prepared within pH 9–13 had higher (*P* < 0.05) TS by about 2.6 ± 0.3 MPa than films prepared within pH 2–4. Measured TS values of WG films within pH 9–13 were fairly well fitted by the regression equation (*R*² = 0.87)

$$TS = 0.011(pH)^{2.36} \qquad (2)$$

The maximum mean TS of WG films (pH 12–13) was higher by about 20% than the maximum mean TS of SPI

films (pH 8–10). This could be partially attributed to the higher amount of plasticizer (60% w/w of protein) contained in SPI films than in WG films (37% w/w of protein). In general, plasticizers function by decreasing accumulation of intermolecular forces along polymer chains, thereby "softening" film structure, decreasing TS, and increasing *E* (Mellan, 1961).

**Percentage Elongation at Break (*E*).** *E* is determined at the point where the film breaks under tensile testing and is expressed as the percentage of change of the original length of the specimen between the grips of the testing machine (Briston, 1988). The ability of a film to stretch (extend) is indicated by *E*. Generally, *E* of SPI films increased as pH was raised above the protein's isoelectric point (Table I). On the average, SPI films from pH 6 to 11 had greater (*P* < 0.05) *E* by about 104 ± 16% than films cast from pH 1 to 3. *E* values of SPI films within pH 6–11 can be reasonably well estimated by the first-order regression equation (*R*² = 0.76)

$$E = -28.1 + (20.2)pH \qquad (3)$$

No significant differences (*P* < 0.05) in *E* were noticed for WG films cast from different pH values (Table II). WG films, although containing less plasticizer, appeared to have higher *E* values than SPI films. However, *E* does not account for film thickness. Consequently, comparisons in terms of *E* are meaningful only for films of practically equal thickness. SPI films produced in this study were substantially thinner (by about 45%) than WG films.

**Water Vapor Permeability (WVP).** WVP is a proportionality constant assumed to be independent of the water vapor pressure gradient applied across a film (Rogers et al., 1962). However, hydrophilic (edible or nonedible) materials, such as protein films, deviate from this ideal behavior due to interactions of permeating water molecules with polar groups in the film structure (Myers et al., 1962; Hagenmaier and Shaw, 1990). For example, WVP varied with applied water vapor pressure gradient for hydrophilic films from cellulosic derivatives (Karel et al., 1959; Woodruff et al., 1972) and from amylose (Rankin et al., 1958). Deviation from the ideal behavior can also be induced by temperature effects on materials (Karel et al., 1959; Myers et al., 1962). WVP of edible films based on lipid materials could also appear to be dependent on water vapor pressure gradient due to clustering of water molecules in the nonpolar film structure. As a result, WVP values reported in the present study apply only to the specific experimental conditions (50% relative humidity gradient and 25 °C). Use of such terms as "effective water vapor permeability constant" (Biquet and Labuza, 1988) or "apparent water vapor permeability constant" (Kester and Fennema, 1989) would also be appropriate.

WVP of SPI films was lower at pH values above the protein's isoelectric point (Table I). On the average, SPI films from pH 6 to 12 had lower (*P* < 0.05) WVP by (1.6 ± 0.5) × 10⁻⁹ g/(m s Pa) than films prepared at acidic conditions (pH 1–3). No significant differences (*P* < 0.05) in WVP were noticed within pH 6–12. The lower TS and higher WVP of SPI films produced at pH 1–3 suggest that less intermolecular protein cross-linking occurs under acidic than alkaline conditions. Most likely, at the highly acidic conditions of pH 1–3, significant repulsive forces develop among positively charged soy protein chains, resulting in films of decreased strength and less dense structure. In contrast, WG films produced at extreme acidic conditions (pH 2–3), as well as at extreme alkaline conditions (pH 13), had the lowest (*P* < 0.05) WVP (Table II).

Table III.  Tensile Strength (TS), Percentage Elongation at Break (*E*), and Water Vapor Permeability (WVP) of Polymeric Films[a]

| film | TS, MPa | *E*, % | WVP,[b] g/(m s Pa) |
|---|---|---|---|
| low-density polyethylene | 8.6–17.3 | 500 | $7.3\text{–}9.7 \times 10^{-13}$ |
| high-density polyethylene | 17.3–34.6 | 300 | $2.4 \times 10^{-13}$ |
| cast propylene | 41.5 | 300 | $4.9 \times 10^{-13}$ |
| poly(vinylidene chloride) | 48.4–138 | 20–40 | $0.7\text{–}2.4 \times 10^{-13}$ |
| cellulose acetate | 48.5–82.7 | 15–45 | $0.5\text{–}1.6 \times 10^{-11}$ |
| ethylene–vinyl acetate | 13.8 | 650–800 | $2.4\text{–}4.9 \times 10^{-12}$ |
| polyester | 178 | 70–100 | $1.2\text{–}1.5 \times 10^{-12}$ |

[a] From Briston (1988). [b] For 25-$\mu$m films at 90% relative humidity gradient and 38 °C.

## Acidic vs Alkaline Conditions.

In agreement with the findings of Gontard et al. (1992), it was noticed that WG films processed under alkaline conditions had a more yellowish coloration than acidic films. However, the visual disadvantage of alkaline films is offset by their significantly higher TS. SPI films of good property characteristics were produced at pH values around neutral, whereas WG films of maximal TS were produced at pH 12–13. Such high film alkalinity is undesirable when films are intended for use in edible packaging. In this situation, use of a volatile base, such as ammonium hydroxide, which volatilizes as ammonia upon drying, in the place of sodium or potassium hydroxide has been recommended (Anker et al., 1972). However, persistence of an ammoniacal odor and, in the case of commercial scaleup, handling of ammonia fumes constitute disadvantages of using ammonium hydroxide. Similarly, a volatile acid (e.g., acetic acid) could be employed for obtaining dried WG films of nearly neutral pH after processing under acidic conditions (Gontard et al., 1992). Protein film alkalinity should not be a major concern in nonedible applications, such as possible use of protein films as high oxygen barriers in multilayer packaging materials (Krochta, 1992; Gennadios et al., 1993a).

SPI film-forming solutions, unlike WG solutions, require no ethanol. This means lower ingredient costs and lack of problems related to handling of evaporating ethanol. On the other hand, less energy (drying heat) is needed to dry the ethanol-containing WG film-forming solutions than the 100% aqueous SPI film-forming solutions because of ethanol's lower boiling point.

## Comparison with Polymeric Films.

Resistance of protein films to water vapor transmission is limited due to the inherent hydrophilicity of proteins. Transmission of water vapor through protein films is also facilitated by the hydrophilic glycerin which favors adsorption of water molecules. Water vapor permeability constant values determined in this study for WG and SPI films were higher by 2–4 orders of magnitude than those of typical polymeric packaging materials (Table III). WG and SPI films had substantially lower tensile strength than polymeric materials, whereas protein film elongation at break was greater than that of poly(vinylidene chloride), cellulose acetate, and polyester (Table III). The large amount of plasticizer (glycerin) added to obtain free-standing protein films played an important role in films having low tensile strength and adequate flexibility.

Possibilities of improving protein film properties by promoting cross-linking through treatment with chemicals (e.g., aldehydes) and enzymes (e.g., transglutaminase) should be explored. However, the toxicity of certain protein cross-linking agents, such as most aldehydes, will limit protein film use to nonedible applications. Water

vapor permeability characteristics of protein films may also be improved through addition of hydrophobic lipid materials.

**Potential Applications.** Although both WG and SPI films are poor water vapor barriers, they have been found to be very effective oxygen barriers (Brandenburg et al., 1993; Gennadios et al., 1993). Consequently, packaging applications that utilize the films' oxygen barrier ability should be primarily sought for WG and SPI films. For example, use of such films as oxygen barrier layers in multilayer packaging materials appears to be feasible. This is analogous to the use of moisture-sensitive ethylene–vinyl alcohol and polyamide films as oxygen barriers in multilayer packages. Several food items susceptible to lipid oxidation could benefit from application of protein coatings in combination with an external moisture barrier. Protective protein coatings could also be used on certain food products, such as meat pies and high-moisture low-sugar cakes, that require films highly permeable to water vapor. Employing protein coatings as carriers of antioxidants and other food additives is another envisioned application.

## ABBREVIATIONS USED

*E*, percentage elongation at break; SPI, soy protein isolate; TS, tensile strength; WVP, water vapor permeability; WVTR, water vapor transmission rate.

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