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Development and Characterization of Films Based on Chemically Cross-Linked Gliadins†

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The aim of the present work has been to study the possibility of obtaining modified gliadin films with improved water resistance and mechanical properties by means of promoting intermolecular covalent bonds between polypeptide chains. Prior to casting films, formaldehyde, glutaraldehyde, and glyoxal were used to cross-link proteins at concentrations ranging from 1% to 4% (grams per 100 g of protein). Mechanical properties (tensile strength and elongation at break), water vapor permeability, moisture sorption isotherms, and optical properties of the films produced were evaluated as a function of the cross-linker used. Experimental results showed that some properties of gliadin films were considerably modified. Cross-linking improved the water resistance of films, avoiding their disintegration. Their water barrier properties were also enhanced, but their moisture sorption properties remained unchanged. Formaldehyde imparted greater mechanical strength to films than glutaraldehyde or glyoxal, increasing tensile strength values 10-fold. Addition of the cross-linkers at concentrations in excess of 2.5% did not further improve the mechanical or barrier properties. However, modification with glutaraldehyde or glyoxal imparted an increasingly yellowish tint to the films.

KEYWORDS: Protein films; gliadins; chemical cross-linking; water vapor permeability; moisture sorption isotherms; mechanical properties

INTRODUCTION

Major efforts are under way in order to find new uses for polymers derived from agricultural sources. Most of these materials present good film-forming properties that make them suitable for packaging, including food packages and agricultural applications such as waste bags, mulching, or soil retention sheeting. Developing eco-friendly materials could overcome social issues concerning environmental pollution associated with the vast use of petrochemical-derived plastic materials. Moreover, nonfood industrial uses of these renewable materials can promote economic development in the agricultural sector and help to preserve limited natural resources.

Among the naturally derived polymers, plant proteins are a good potential resource for the production of plastic films. Several reviews can be found on the use of plant proteins such as corn zein, soy, and wheat gluten in nonfood applications (*1*−*3*). Protein films are of moderate physical strength compared to synthetic films, and the use of a plasticizer is required to overcome film brittleness. They are also good oxygen barriers in low and intermediate relative humidity environments compared to polyolefins (*3*). One of the major limitations of proteins as packaging materials is their high water sensitivity, which is associated with the inherently hydrophilic nature of these macromolecules.

Proteins have a large number of reactive side groups susceptible to physical, chemical, or enzymic modifications, thus making it possible to obtain appropriate functional properties depending on the purpose intended. In this regard, many enzymatic and physical modifications of proteins (e.g., heating and high-pressure processing) have been described to be useful for conferring surface activities and textural attributes to foods (*4*).

Covalent cross-linking of polypeptide chains is a valuable mechanism for increasing the strength of tridimensional protein networks and providing greater physical integrity in aqueous media. The cystine bond is the most ubiquitous cross-link present in native proteins. Disulfide- or sulfhydryl-containing proteins can undergo sulfydryl−disulfide interchange reactions under specific environmental conditions, introducing new intra- and intermolecular cross-links and therefore resulting in structural network changes (*5*). Specific enzymes can also act as covalent cross-linkers. Among them, transglutaminase, which catalyzes the formation of an ϵ-(γ-glutamyl)lysine bond, is

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widely employed for producing covalently cross-linked gels from a wide range of food protein types (*6*).

Cross-linking of protein-based films is very well suited to improving specific characteristics required in packaging applications such as water resistance and mechanical integrity. Transglutaminase has been employed in the formation of cross-linked films from proteins (*7*). Ultraviolet radiation treatments of soy proteins, sodium caseinates, wheat gluten, corn zein, and egg albumen proteins, γ-irradiation of soy proteins, sodium caseinates, and whey protein isolates, and the application of ultrasound frequency to whey protein concentrate have all modified the properties of the derived films (*8*−*10*). Controlled thermal treatments have been effective in cross-linking protein films (*11*). The promotion of disulfide bridging through cysteine treatment or heating has also resulted in wheat gluten-derived films with improved mechanical and water barrier properties (*12*, *13*). Naturally occurring cross-linkers such as genipin and tannic acid have been reported to modify the mechanical and water barrier properties of protein films (*14*).

Proteins intended for nonfood applications are able to be cross-linked by a broad variety of chemical agents. Bifunctional and multifunctional reagents such as diisocyanates and carbodiimides have recently been used to improved functional properties of films made from keratin, wheat gluten, and zein (*15*−*17*). Diisocyanates act as lysine-targeted cross-linkers, and carbodiimides selectively link carboxylic acid and amine groups. Formaldehyde has the broadest reaction specificity, being able to cross-link not only the ϵ-amine group of lysine but also the side chains of the amino acids cysteine, tyrosine, histidine, tryptophan, and arginine, thus promoting the formation of intra- and intermolecular covalent bonds (*18*), α,ω-Dialdehydes are extensively used as cross-linkers in proteins. Glutaraldehyde is more specific than formaldehyde, reacting with lysine, cysteine, histidine and tyrosine. Lysine and arginine residues are potential targets for glyoxal (*19*).

Formaldehdye, glutaraldehyde, and glyoxal have been used by several authors to enhance the functionality of soy protein, collagen, cottonseed protein, corn zein, sunflower protein isolate, gelatin, and peanut protein films intended for food packaging applications (*20*−*27*).

Gluten is a biodegradable and renewable material with noteworthy potential for technological applications. Nonfood uses of gluten such as plastics are related directly to its adhesive, cohesive, elastic, and low water solubility properties (*28*). Previous studies undertaken on gluten proteins have shown that gliadins and glutenins can be exploited separately to maximize their range of industrial uses (*29*). Films made from gliadins are very glossy and transparent but have poor mechanical resistance and they disintegrate upon immersion in water, which limits their application as a packaging material.

The aim of this work has been to obtain water-resistant gliadin films having improved mechanical properties by treatment of proteins with formaldehyde, glutaraldehyde, and glyoxal prior to film manufacture. The effect of protein modification on specific properties of the derived films were studied and compared in terms of the cross-linker employed.

MATERIALS AND METHODS

Reagents. Crude gluten from wheat (80% protein, 7% fat, and 8.1% moisture content on a dry basis), glycerol, ethanol, acetic acid, and the cross-linking agents formaldehyde (FA) (37% solution), glutaraldehyde (GTA) (50% solution), and glyoxal (GLY) (40% solution), all laboratory grade, were supplied from Sigma Co. (St. Louis, MO).

Gliadin-Rich Fraction Extraction from Wheat Gluten. Crude wheat gluten (100 g) was dispersed in 400 mL of 70% (v/v) aqueous ethanol at pH 6.75 (*30*). After the mixture was stirred overnight, proteins were centrifuged at 3700 rpm for 30 min at 5 °C. The resulting supernatant containing the gliadin-rich fraction was collected and used as the gliadin film-forming solution. The precipitate consisting of glutenins and residual starch was discarded. Protein content was determined by the micro-Kjeldahl method 46-13 (*31*) after previous evaporation of the solvent. Gliadin recovery was 34% (grams per 100 g of gluten).

Chemical Modification of Gliadins. Protein cross-linking was conducted by adding FA, GTA, or GLY at 1%, 2.5%, and 4% (grams of cross-linking agent/100 g of protein) in the film-forming solution at 23 °C for 1 h under gentle stirring. Preliminary studies carried out showed that incubation times longer than 1 h did not modify the functional properties of films.

Film Formation and Conditioning. Glycerol was added as plasticizer to the protein film-forming solution at 33% (grams per 100 g of dry protein). Films used for the thermal analysis and the determination of the moisture sorption isotherms were made without adding any glycerol to the film-forming solution. Film-forming solution (40 g) was poured onto a horizontal flat Teflon tray (37×24 cm^2), and water and ethanol were allowed to evaporate. The films were dried at 23 ± 2 °C and $50\% \pm 5\%$ relative humidity for 10 h. Dry films were peeled from the casting surface and preconditioned in a chamber at 23 ± 2 °C and $50\% \pm 5\%$ relative humidity for at least 72 h prior to testing.

Film Thickness. Film thickness was measured by use of a micrometer (Fisher Scientific, Pittsburgh, PA) with a sensitivity of ± 2.54 μm. Mean thickness was calculated from measurements taken at five different locations on each film sample. Average thickness of the samples was 55 ± 5 μm.

Differential Scanning Calorimetry. Measurements of the glass transition temperature (T_g) of the films made without addition of glycerol were performed with a differential scanning calorimeter (TA DSC 2920, TA Instruments Inc., New Castle, DE) equipped with an Universal V2.6B TA integrator. The instrument was calibrated with indium as a standard. Films were conditioned over P_2O_5 at 23 °C for 2 weeks before testing. Dry samples of 8 mg were placed in a hermetically sealed aluminum pan and heated at 5 °C/min from 23 to 190 °C under a nitrogen flow (50 mL/min). It is known that wheat glutenin presents an endothermic relaxation peak superimposed on the glass transition phenomenon (*32*). As the glass transition is a reversible phenomenon, a first scanning was performed to eliminate this relaxation and a second scanning was run after quick cooling to reveal the T_g value. T_g was recorded as the midpoint temperature of shift in the baseline due to change in heat capacity upon glass transition. Specimens were measured in duplicate.

Moisture Sorption Isotherms. Moisture sorption isotherms were evaluated for the control and chemically treated films made without addition of glycerol. Films were cut into small pieces of 4−5 g, placed in aluminum dishes, and allowed to reach moisture equilibrium with eight different salt solutions in 10 L sealed containers placed in an environmental chamber conditioned at 25 °C. The relative humidity values in the containers were $11.3\% \pm 0.3\%$, $22.5\% \pm 0.3\%$, $32.8\% \pm 0.2\%$, $43.2\% \pm 0.4\%$, $52.9\% \pm 0.2\%$, $75.3\% \pm 0.1\%$, and $93.6\% \pm 0.2\%$ (ASTM E 104-85) (*33*). We assumed equilibrium was reached when the change in sample mass was less than 1%. Equilibrium moisture content was determined by drying the samples in a vacuum oven at 60 °C for 24 h (*34*). The moisture content was calculated on a dry basis and reported as the average of three replicates at each relative humidity condition.

Water Vapor Permeability. Water vapor transmission rate [grams per (square meter·second)] through the films was measured on a Permatran W3/30 (MoCon Inc., Minneapolis, MN) at 23 °C with a gradient of 50% relative humidity to 0% relative humidity (dry nitrogen) across the film. At least three specimens of each type of film produced were measured. Permeability values are reported as water vapor permeability coefficient in (gram·meter)/(square meter·second·pascal).

Weight Loss. Film specimens were dried in a desiccator containing dry calcium sulfate. Dry film samples of about 500 mg were immersed in beakers containing 50 mL of distilled water at 23 °C during 24 h with periodical gentle manual agitation. Films were removed from the water and replaced in the desiccator until they reached a constant weight

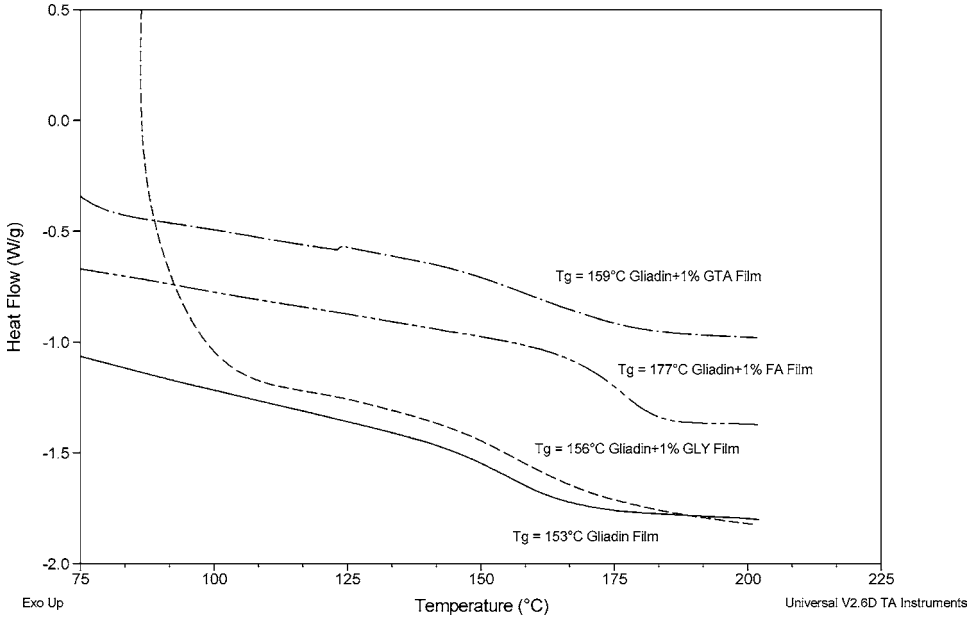

Figure 1. DSC thermogram of control and films cross-linked with 1% GLY, 1% GTA, and 1% FA.

in order to obtain the final dry weight of the film. The percentage of weight loss of the films in water (% WL) was calculated from

$$\%\ \mathrm{WL} = [(\text{initial dry weight} - \text{final dry weight})/\text{initial dry weight}] \times 100 \quad (1)$$

WL tests for each type of film were performed in triplicate.

Mechanical Properties. An Instron Universal Machine Model 4201 (Canton, OH) equipped with a 1 kN static load cell was used to evaluate the tensile strength (TS) and percentage elongation at break (% E) of films according to the ASTM standard D-882-3 (*35*). Film samples were cut into strips 25.4 mm wide and at least 10 cm long. The Instron grip separation was set at 50.8 mm, with cross head speed 508 mm/min. TS values were reported in pascals, and % E values were in percent of increase in length divided by initial grip separation. Mechanical properties were measured in a room conditioned at 23 °C and 50% ± 5% relative humidity. At least 10 samples from each type of film were evaluated.

Film Color. Film color was determined with a hand-held Minolta Chroma Meter CR300 (Minolta Camera Co., Ltd., Osaka, Japan) set to D65 illuminant/10° observer. Film specimens were placed on the surface of a white standard plate and the CIELAB color space was used to determine the parameters: L^* (0 black to 100 white), a^* (− greenness to + redness), and b^*(− blueness to + yellowness). Color can also be expressed by use of polar coordinates L^*, C^*, and H^*, where L^* is the same as previously, C^* is chroma or saturation index, and H^* is hue. Simple transforms are used to convert $L^*a^*b^*$ coordinates to $L^*C^*H^*$ coordinates:

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

$$H^* = \arctan(b^*/a^*) \quad (3)$$

Five measurements were taken of each sample and three samples of each film were measured. Films were conditioned at 50% relative humidity for 72 h prior to testing.

Statistical Analysis. Statistical analysis of the results was performed with SPSS commercial software (SPSS Inc., Chicago, IL.). A one-way analysis of variance (ANOVA) followed by a Tukey test ($p < 0.05$) was used to identify which film means differed significantly. The data were analyzed and graphically plotted with SigmaPlot software (Systat Software Inc., Richmond, CA.).

RESULTS AND DISCUSSION

DSC. Gluten proteins are amorphous random biopolymers capable of undergoing a glass transition from a rubberlike to a glassy state. The mechanical and rheological behavior of these biopolymers is considerably modified at T_g and affects the final properties of the films derived. Apart from chemical structure and the presence of added plasticizers, the T_g of polymers is governed by other structural features such as molecular weight, chain branching, crystallinity, and extent of cross-linking. **Figure 1** shows thermograms for dry films made from unplasticized gliadins and those made from gliadins modified with FA, GTA, or GLY at a concentration of 1%. Films were made without glycerol since the aim of the thermal analysis was to observe whether cross-linking imparts some modification on T_g of proteins cast into films without any additional effects of the plasticizer or moisture. As can be seen in **Figure 1**, the T_g of gliadin film had a value of 153 °C, thus falling within the range of values obtained by several authors for native gliadins (*32*, *36*, *37*). After modification of gliadins with aldehydes, the T_g of the films shifts to higher values as expected since cross-links restrain polymer chain mobility, which increases T_g. T_g of films made from proteins treated with FA showed a greater increase than those treated with GTA or GLY. FA is able to react with a larger variety of functional groups in gliadins than GTA or GLY, implying the generation of a greater density of covalent cross-links in the protein network and therefore a higher T_g for the film. This phenomenon has been studied to a lesser extent for proteins: Ustunol et al. (*38*) reported an increase in the T_g of whey protein films modified with FA or GTA. Cross-linking of sorghum karifin with tannic acid and sorghum-condensed-tannins also increased T_g of the resulting films (*14*). T_g of gelatin microspheres increased upon chemical modification by GTA (*39*).

Moisture Sorption Isotherms. Moisture sorption isotherms of the control and films made from proteins cross-linked in the absence of glycerol are shown in **Figure 2**. Treated and untreated films showed similar moisture sorption isotherms, a result also observed for gelatin films cross-linked with transglutaminase, glyoxal, or formaldehyde (*28*). The isotherms are sigmoidal in shape, corresponding to type II in the Brunauer−Emmett−Teller (BET) isotherm classification. Three regions can be observed in the isotherms. The first region of the curve is convex and corresponds to the monolayer adsorption of water molecules to polar sites in the film via hydrogen bonds; the

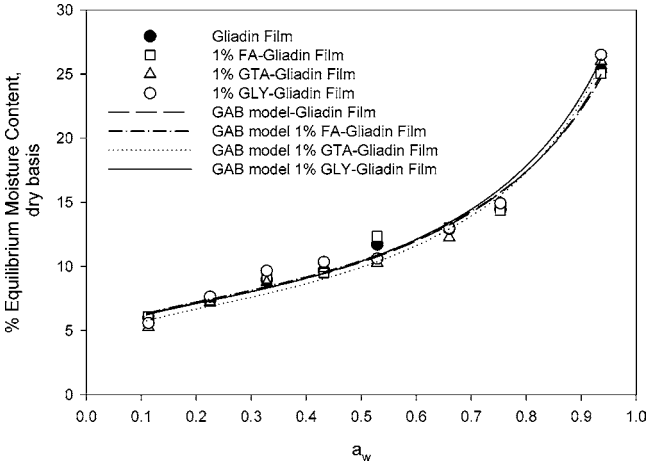

Figure 2. Experimental moisture sorption isotherms at 23 °C and curves predicted by the GAB model.

linear region of the isotherms indicates that water is adsorbed as a multilayer; the upper part of the curve corresponding to water activities above 0.6 is characterized by a sharp upturn reflecting swelling of the polymer and the presence of water molecules in a bulk liquid phase. This multilayer sorption model is commonly encountered with proteins and other hydrophilic macromolecules. Cross-linking does not appear to change the moisture sorption behavior of protein over the range of water activities evaluated (11.3−93.6%). The moisture sorption isotherms were found to be well described by the Guggenheim−Anderson−deBöer (GAB) model, which is one of the most extensively used for food materials and naturally occurring biopolymers. This model is an extension of the BET model and adequately describes moisture sorption isotherms for water activities up to about 0.9:

$$\mathrm{EMC} = \frac{W_m C k a_w}{(1 - k a_w)(1 - k a_w + C k a_w)} \quad (4)$$

where EMC = equilibrium moisture content on a dry basis, W_m = BET monolayer moisture content and represents water content corresponding to the saturation of all primary adsorption sites by one water molecule, C = Guggenheim constant, k = factor correcting properties of the multilayer molecules corresponding to the bulk liquid, and a_w = water activity. The GAB equation can be rearranged to a polynomial expression according to Bizot (*40*):

$$\frac{a_w}{\mathrm{EMC}} = \alpha a_w^{\,2} + \beta a_w + \gamma \quad (5)$$

where

$$\alpha = \frac{k}{w_m}\left[\frac{1}{C} - 1\right] \quad (6)$$

$$\beta = \frac{1}{w_m}\left[1 - \frac{2}{C}\right] \quad (7)$$

$$\gamma = \frac{1}{w_m C k} \quad (8)$$

α, β, and γ can be obtained by a nonlinear regression analysis of a_w/EMC vs a_w.

Table 1. Guggenheim–Anderson–deBöer Model Constants and Root Mean Square (RMS) of the Fitting for Control and Films Treated with FA, GTA, or GLY at 1%

film	W_m	C	k	RMS, %
gliadin	6.3	111	0.80	0.69
gliadin + 1% FA	6.4	95	0.79	1.42
gliadin + 1% GTA	6.0	71	0.82	3.80
gliadin + 1% GLY	6.3	98	0.81	4.44

The "root-mean-square" (RMS, %) of the fitting was calculated for each film:

$$\%\ \mathrm{RMS} = \left[\sqrt{\frac{\sum\left[\frac{M^{\exp} - M^{\mathrm{calc}}}{M^{\exp}}\right]^2}{N}}\right] \times 100 \quad (9)$$

where N is the number of experimental points, $M^{\exp}$ is the experimental equilibrium moisture content value, and M^{calc} is the calculated equilibrium moisture content value.

Constants for the model are given in **Table 1**. Monolayer moisture content values of control and modified films were similar. It could be expected that cross-linking would reduce the number of specific water sorption sites and thus the BET monolayer value. However, cross-linking of proteins in solution can increase the accessibility of groups susceptible to chemical modification that are not available as sorption sites for water molecules in the final film. BET monolayer values for gliadin films are in the range found for other proteins (*41*). C and k constants shown in **Table 1** were within the ranges $0.24 < k \leq 1$ and $5.67 \leq C \leq \infty$, respectively, given by Lewicki (*42*) to yield a relatively good description of the sigmoidal type of the isotherm and to fulfill the requirements of the BET model. Values of k obtained for the films were less than 1, indicating a less structured state of the sorbate in the layers above the monolayer than in the sorbate in the GAB layer. High values of k are found for proteins ($k \approx 0.78-0.85$) and electrolytic systems ($k \approx 0.92$) (*43*). The constant C is related to the difference between the heat of sorption of the first monolayer of water molecules and that of the upper layers. The value of C decreased after cross-linking with FA, GTA, or GLY. There is no clear physical explanation for this behavior since there is no established correlation between a measurable physical parameter and the value of this constant.

For water activities above 0.65 there is a steep increase in the slope of the isotherm, suggesting the existence of moisture as unbound water. It is stated that for hydrophilic films clustering may be expected if the number of water molecules is greater than the quantity that can be bound to the polymer. The range of water activities in which the self-association of water molecules occurs can be determined from an isotherm sorption model such as the GAB equation by applying the clustering function developed by Zimm and Lundberg (*44*). The clustering function is written as

$$\frac{G_{11}}{V_1} = -(1 - \phi_1)\frac{\partial(a_w/\phi_1)}{\partial a_w} - 1 \quad (10)$$

where Φ_1 is the volume fraction of the solute. When G_{11}/V_1 is greater than -1, water is expected to cluster.

The function

$$1 + \phi_1 \frac{G_{11}}{V_1} \quad (11)$$

represents a measure of the cluster dimension. A value greater than 1 indicates the formation of water aggregates.

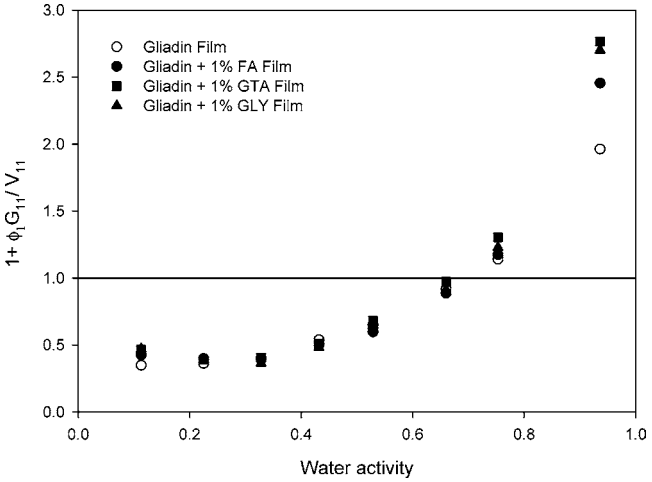

Figure 3. Values of $1 + \Phi_1 G_{11}/V_1$ shown as a function of water activity at 23 °C.

Table 2. Parameters[a] for Gliadin-Rich Films Plasticized with 33% Glycerol at Different Concentrations of Cross-Linker

treatment	TS (MPa)	% E	% LW (g/100 g of dry matter)	WVP × 10^{11} [(g m)/(m^2 s Pa)]
control	0.6 ± 0.1	370 ± 47	*b*	6.8 ± 0.5
1% FA	6.7 ± 0.6	261 ± 22	31.2 ± 0.8	5.5 ± 0.2
2.5% FA	7.1 ± 0.3	250 ± 24	30.1 ± 0.5	5.3 ± 0.3
4% FA	6.5 ± 0.7	250 ± 29	29.5 ± 0.3	5.2 ± 0.3
1% GTA	2.5 ± 0.2	268 ± 38	32.8 ± 0.6	5.5 ± 0.2
2.5% GTA	2.7 ± 0.4	261 ± 22	32.5 ± 1.0	5.6 ± 0.3
4% GTA	2.8 ± 0.8	245 ± 18	31.5 ± 0.5	5.3 ± 0.2
1% GLY	1.9 ± 0.2	256 ± 39	29.5 ± 1.0	5.7 ± 0.2
2.5% GLY	2.1 ± 0.2	223 ± 37	31.0 ± 1.0	5.4 ± 0.2
4% GLY	2.1 ± 0.2	234 ± 37	30.7 ± 1.0	5.2 ± 0.4

[a] TS, tensile strength; % E, percentage of elongation at break; WL, loss of weight after immersion in water; WVP, water vapor permeability. [b] Disintegrated.

In **Figure 3** values of the cluster dimension are reported as a function of water activity. A minimum value for the function can be observed at $a_w \approx 0.3$, with steadily increasing values at intermediate humidities. Water molecules tend to cluster for a_w greater than ≈0.65 for control and modified films. There is little work in the literature regarding water sorption and water clustering in proteins (*45*, *46*). As water activity increases, the average dimension of water clusters increases considerably. In this region of the curve (**Figure 3**) it can be appreciated that the function reaches the highest values for cross-linked films as compared to the control. This behavior has also been found for collagen films cross-linked with FA or chrome (*46*). A possible explanation for this phenomenon is that the swelling of the protein matrix at high relative humidities facilitates the exposure of water sorption sites in unmodified gliadins that are not available after cross-linking.

Water Vapor Permeability. Chemical cross-linking of gliadins with FA, GTA, or GLY enhanced the moisture barrier properties of the derived films. WVP values were decreased by around 25% in all the treatments with aldehydes without significant differences among them ($P < 0.05$). Amounts of cross-linker greater than 1.5% did not modify permeability values (**Table 2**). Permeability through nonporous membranes is governed by a two-stage mechanism involving thermodynamic dissolution of permeant molecules into the solid polymer network at the high-pressure face and subsequent kinetic diffusion through the film. Thus, barrier properties of polymeric materials are determined by the chemical structure of the chain and system morphology (*47*). As has been mentioned above, the solubility of water vapor molecules is not apparently influenced by incorporation of intermolecular covalent bonds in the protein network. This implies that the decreases in WVP values after protein modification are likely to be due to differences in structural features present in the protein network. The development of permanent covalent bonds between polypeptide chains by means of low molecular weight aldehydes promotes the formation of protein aggregates and restricts the ability of segment polymer chains to relax and shift their structure. As a result, the formation of temporal voids essential for activated diffusion is limited and the resulting permeation of water vapor molecules through the film tends to decrease. Although there are a significant number of papers regarding the effect of cross-linking on mechanical properties of biopolymers, water vapor permeability studies are scarce. Water vapor permeability of films made from soy protein isolate (*20*), gelatin (*26*), peanut protein (*27*), and whey protein isolate (*38*) decreased after treatment with FA or GTA.

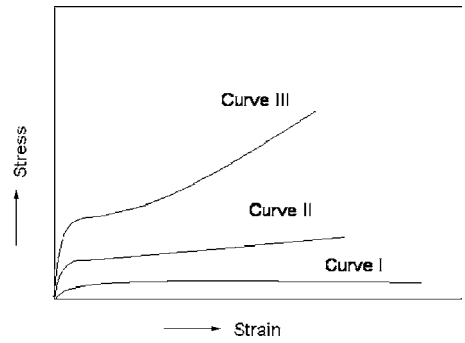

Figure 4. Stress–strain curves for control (curve I) and films cross-linked with GTA (curve II) and FA (curve III).

Weight Loss. Values of weight loss of films after immersion in water at 23 °C for 24 h are presented in **Table 2**. Gliadins are poorly soluble in water, and although films formed from these proteins without any chemical treatment were not soluble, they disintegrated after immersion in water. Films obtained from gliadins chemically modified with FA, GTA, or GLY maintained their integrity in water. As can be observed in **Table 2**, the loss of weight of cross-linked films was around 30% and this was not significantly altered by aldehyde concentrations above 1% ($p > 0.05$). The gliadin network presents a high density of intermolecular secondary bonding forces such as hydrogen and hydrophobic interactions, which are generally much weaker than covalent bonds. Disulfide linkages, the most significant covalent bonds in cereal proteins, are present in gliadins only intramolecularly. Water causes unbonding of the polymeric chains from the matrix and disintegration of the film. Introduction of covalent bonds by means of aldehydes leads to the formation of a permanent cross-linked network resistant to water. FA cross-linked whey protein and soy protein films reduced substantially their solubility in water (*20*, *38*). FA and GTA imparted similar reduction on water solubility of peanut protein films (*27*).

The loss of weight of the cross-linked films corresponds to the glycerol added as a plasticizer, which diffuses into the water. This explanation was corroborated by the fact that the films immersed in water become very brittle after drying and conditioning at 50% RH.

Mechanical Properties. Figure 4 shows stress−strain curves of films obtained at 23 °C and 50% relative humidity. Curve I corresponds to gliadin control films plasticized with 33% glycerol. Films present uniform viscous behavior with low tensile strength and high extensibility, corresponding to a very soft rubbery material. In other studies it has been observed that

the addition of gliadins to bread dough decreases the rupture viscosity and increases the rupture strain of the dough. On the basis of dough rheological studies, it is widely accepted that gliadins are responsible for dough extensibility (viscosity) while glutenins provide strength (elasticity) (*48*). This correlates with the observed behavior of untreated gliadin film. Gliadins modified with FA (curve III) or with GTA and GLY (curve II) gave rise to stiffer films with increasing fracture stress and lower extensibility. By comparison of curves II and III, it can be seen that films formed from proteins subjected to FA treatment were stiffer and stronger than those prepared from GTA or GLY-modified gliadins. It is worth noting that films modified with aldehydes experienced strain hardening as revealed by an increase in the slope of the strain−stress curve with increasing extension just above the yield point. This increase was greater for films made from FA-treated gliadins. Gluten and dough both exhibited strain hardening, which is thought to arise mainly from entanglement coupling of the larger glutenin molecules (*49*). In relation to gliadins, the creation of new constraints on chain mobility and the formation of aggregates due to chemical cross-linking could explain the strain hardening of the plasticized films. Gallstedt et al. (*50*) have recently reported that gluten films molded at high temperature show increased strain hardening; it is well-known that temperature is another means of cross-linking disulfide-containing proteins. Tensile strength and elongation at break values of films are given in **Table 2**. As can be observed, aldehyde concentrations in excess of 1% (2.5% and 4%) did not have any additional effect on the mechanical properties of the films. All the treatments with aldehydes increased tensile strength and decreased extensibility compared to the control. By comparison of the effects of the different aldehydes under study, FA provided more mechanically resistant films than GTA or GLY, resulting in an approximately 11-fold increase in TS values. Films from GTA- and GLY-modified gliadins exhibited 4.5- and 3.5-fold increase, respectively. The greater efficiency of FA in strengthening the films can be explained by the lack of specificity of this chemical with respect to the different amino acid side-chain groups it reacts with. GTA imparted slightly higher TS values to films than GLY ($p < 0.05$). These results are in agreement with other studies carried out on protein films. Marquié et al. (*22*) found that FA was more effective than GTA at enhancing the puncture strength of films made from cottonseed proteins. Comparing the effectiveness of FA and GLY as cross-linkers, Carvalho et al. (*26*) reported that gelatin films achieved greater tensile strength values when cross-linked with FA.

Table 2 shows that elongation at break was reduced by approximately 30% after chemical treatment with aldehydes without significant differences between treatments ($p > 0.05$). Despite the formation of a more rigid structure after cross-linking, films conserved good flexibility and handling. When our results are compared with those reported in the literature for wheat protein films post-treated with FA vapors (*51*), it is noteworthy that the extensibility of our films decreased to a lesser extent but achieved similar tensile strength values. This observation may be explained by considering the different physical natures of the substrates. In a film, the distribution of cross-links will be determined by the structural restrictions imposed by the already formed network of protein molecules. In solution, such restrictions are not present and the architecture of the protein network will be dictated at least in part by the cross-links randomly formed in the soluble phase. Thus, cross-linking of proteins in solution could lead to reduced chain-junction entangling when the solvent is removed and the chains collapse (*52*), hence explaining greater flexibility observed in films cast from pretreated (cross-linked) proteins.

Table 3. Effect of Glycerol on *L**, *a**, and *b** Coordinates of Films

treatment	*L**	*a**	*b**
control	97.3 ± 0.7	–0.21 ± 0.04	4.5 ± 0.3
1% FA	97.7 ± 0.4	–0.17 ± 0.03	4.0 ± 0.2
2.5% FA	97.6 ± 0.3	–0.15 ± 0.03	3.4 ± 0.1
4% FA	97.8 ± 0.4	–0.14 ± 0.04	3.7 ± 0.3
1% GTA	95.8 ± 0.4	0.79 ± 0.05	8.3 ± 0.6
2.5% GTA	95.6 ± 0.2	0.92 ± 0.01	8.9 ± 0.2
4% GTA	95.1 ± 0.7	0.95 ± 0.06	11.8 ± 1.1
1% GLY	96.7 ± 0.3	0.90 ± 0.04	7.7 ± 0.5
2.5% GLY	96.4 ± 0.4	0.96 ± 0.06	8.4 ± 0.2
4% GLY	96.4 ± 0.3	1.06 ± 0.03	9.1 ± 0.6

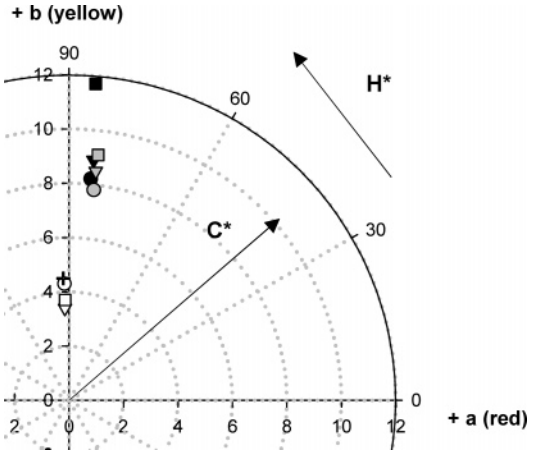

Figure 5. L*C*H* polar diagram. Control film (+), FA–gliadin films (white symbols), GTA–gliadin films (black symbols), and GLY–gliadin films (gray symbols) at different concentrations of cross-linker (circles, 1%; triangles, 2.5%; squares, 4%) are shown.

Film Color. *L**, *a**, and *b** color coordinates of films are shown in **Table 3** and chroma (C^*_{ab}) and hue (H^*_{ab}) are plotted in a polar diagram in **Figure 5**. Films made from gliadins were very transparent as indicated by *L** and acquired a slight yellowish color due to the natural presence of carotenoids and flavonoids in wheat. Lightness of films made from proteins modified with FA increased relative to the control but differences were not significant ($p > 0.05$), while the *a** value increased and the *b** value decreased significantly ($p < 0.05$). Proteins modified with GTA or GLY gave rise to darker films ($p < 0.05$); *a** and *b** color values also increased significantly ($p < 0.05$). With regard to the effect of aldehyde concentration on film color coordinates, it can be observed that amounts of FA greater than 1% did not exert any effect on lightness of the films but slightly higher values of *a** and lower values of *b** were achieved. Films treated with GTA and GLY exhibited a marked increase in *a** and *b** for concentrations of reagent above 1%. In **Figure 5** it can be observed that GTA and GLY increased the vividness of the films, imparting a yellow-brownish coloration. This color change in proteins has been reported in the literature to be a consequence of the formation of a Schiff base aldimine adduct (CH═N) between the free amino groups of proteins and dialdehydes (*53*). The bleaching effect of FA has also been observed for peanut (*27*) and soy protein films (*20*).

In summary, if the functional properties of gliadins can be enhanced by chemical modification, these proteins could be used as films or coatings for multiple purposes such as agricultural packaging and mulching. However, given the toxicity of the aldehydes, additional studies on the application of naturally

occurring alternative chemical agents able to modify protein film functionality are being carried out.

ABBREVIATIONS USED

FA, formaldehyde; GTA, glutaraldehyde; GLY, glyoxal; DSC, differential scanning calorimetry; WL, weight loss; WVP, water vapor permeability; TS, tensile strength; % E, elongation at break; T_g, glass transition temperature; RH, relative humidity.

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