Characterization of Biodegradable Films Obtained from Cysteine-Mediated Polymerized Gliadins[‡]

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This study focuses on the effect exerted by interchain disulfide bonds on the functional properties of films made from gliadins when cross-linked with cysteine. Gliadins were extracted from commercial wheat gluten with 70% aqueous ethanol, and cysteine was added to the film-forming solution to promote cross-linking between protein chains. The formation of interchain disulfide bonds was assessed by SDS-PAGE analysis. Gliadin films treated with cysteine maintain their integrity in water and become less extensible while their tensile strength increases as a consequence of the development of a more rigid network. The glass transition temperature of cross-linked films shifts to slightly higher values. The plasticizing effects of glycerol and moisture are also demonstrated. The mechanical behavior of cysteine-cross-linked gliadin films was compared to that of polymeric glutenins. Cross-linked gliadins displayed tensile strength values similar to those of glutenin films but achieved slightly lower elongation values. Cysteine-cross-linked gliadin films present the advantage that they are ethanol soluble, facilitating film fabrication or their application as a coating for food or for any other film or surface.

KEYWORDS: Gliadin films; cysteine; disulfide bonds; mechanical properties

INTRODUCTION

There is increasing interest in the use of protein-rich materials as sources of films for agricultural and food-packaging applications. Among the most attractive attributes of these materials for industry and society are their short-time renewable nature, the preservation of fossil-based raw materials, complete biological biodegradability, reduction of the volume of garbage and compostability in the natural cycle, and protection of the climate through the reduction of carbon dioxide release, as well as possible applications for agricultural resources in the production of bio/green materials (*1*). The potential nonfood industrial uses of various proteins derived from plant and animal sources such as wheat gluten, soy, corn zein, whey, casein, albumin, collagen, and gelatin have been reviewed by several authors (*2−6*).

Wheat continues to be one of the world's most important crops in terms of food production. Of the top 30 crops, ~23.4% of this production comes from wheat, followed by corn (21.5%) and rice (16.5%) (*7*). Gluten proteins comprise ~80−85% of total wheat protein and are extremely important from a technological point of view. They are promising biomaterials because they can be transformed into films and, in contrast with other proteins, show very low solubility in water owing to their low content of ionizable amino acid side chains and high content of nonpolar amino acids.

Gluten proteins can be classified in two groups according to their extractability in aqueous ethanol (*8*): glutenins and gliadins. Glutenins are composed of low and high molecular weight (LMW and HMW) subunits and are considered to be among the largest naturally produced protein macromolecules (*9*). Glutenins are capable of forming both intra- and intermolecular disulfide bonds. Intermolecular disulfide bonds can be found between HMW subunits, and between HMW and LMW subunits. HMW subunits form a large disulfide-bonded polymer network with branched LMW subunits. Gliadins are low molecular weight single-chain proteins readily soluble in aqueous ethanol. They have been classified traditionally into four groups, α, β, γ, and ω, on the basis of their electrophoretic mobilities at low pH (*10*). α-, β-, and γ-gliadins are sulfur-rich proteins and contain only intrachain disulfide bonds. In contrast, ω-gliadins lack cysteine residues. According to the Belton "train and loop" model for gluten, hydrogen bonding between the repeat regions of the HMW glutenin subunits is responsible for the elasticity of gluten, whereas gliadins are considered to contribute to gluten viscosity (*11*, *12*).

* Author to whom correspondence should be addressed (e-mail phernan@iata.csic.es; fax 34 96 36 36 301).
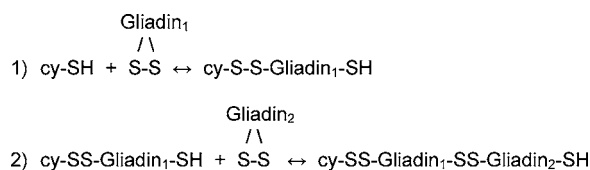‡ This work is dedicated to the memory of Prof. Ruben Hernandez (School of Packaging, Michigan State University) in whose laboratory part of this work was conducted.
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Cysteine residues play an important role in the structure of gluten proteins because they are involved in the formation of both inter- and intramolecular disulfide bonds. Owing to the reducing nature of this amino acid, it could cleave intrachain disulfide bonds, hence promoting molecular rearrangements via disulfide/sulfhydryl exchange reactions. Thus, partial unfolding and the formation of new intermolecular bonds between single-chain gliadins could take place as proposed in the following reactions, where cy-SH represents cysteine:

$$\text{Gliadin}_1$$
$$/\ \backslash$$
1) cy-SH + S-S ↔ cy-S-S-Gliadin₁-SH

$$\text{Gliadin}_2$$
$$/\ \backslash$$
2) cy-SS-Gliadin₁-SH + S-S ↔ cy-SS-Gliadin₁-SS-Gliadin₂-SH

The aim of the current study is to promote the formation of intermolecular bonds between monomeric gliadins by cross-linking with cysteine to obtain a novel ethanol soluble protein network from which can be cast water-resistant films of improved tensile strength. The thermal and mechanical properties of the new gliadin cross-linked films have been compared with those of gliadin and glutenin films.

MATERIALS AND METHODS

Reagents. Crude gluten from wheat (80% protein, 7% fat, and 8.1% moisture content on a dry basis), glycerol, ethanol, L-cysteine, and SDS-PAGE reagents were supplied by Sigma Chemical Co. (St. Louis, MO).

Film Formation. Gliadins and glutenins were extracted from wheat gluten according to a previously described method (*13*). Briefly, 100 g of crude wheat gluten was dispersed in 400 mL of 70% (v/v) aqueous ethanol, stirred overnight at room temperature, and centrifuged at 10000g for 30 min at 23 °C. The resulting supernatant containing the gliadin-rich fraction was collected and used as the gliadin film-forming solution. The precipitate, consisting mostly of glutenins, was resuspended in a solution of 50% (v/v) ethanol/water and 0.05 N acetic acid and the mixture stirred at 40 °C for 1 h. The insoluble portion containing starch and protein aggregates was eliminated by centrifugation at 10000*g* for 10 min at 23 °C. Glutenins were separated from residual gliadins remaining in the supernatant by precipitation with ethanol. Ethanol was added to a final concentration of 70% (v/v) and the mixture left for 12 h at 2 °C. The precipitated glutenins were obtained by centrifugation at 10000*g* for 30 min at 23 °C and dispersed in 50% (v/v) ethanol/water, yielding the glutenin film-forming solution. The pH of both glutenin and gliadin fractions was reduced to 5 using acetic acid. The initial protein contents of the gliadin and glutenin-rich fractions were 34 and 18.5% (grams per 100 g of gluten), respectively, which was later adjusted to 7.5% (w/w) in the film-forming solution. Protein content was determined using the micro-Kjeldahl method (*14*) after previous evaporation of the solvent.

Chemical modification of gliadins was conducted by adding cysteine to 2% (grams per 100 g of dry protein) in the film-forming solution and incubation at 40 °C for 30 min with gentle stirring. Preliminary studies showed that neither addition of a higher percentage of cysteine nor longer incubation times modified the final properties of the resulting films.

Films were made from gliadins, cross-linked gliadins, and glutenins. Addition of glycerol as a plasticizer was required to impart flexibility to the films and avoid cracking when being handled. Glycerol was added to the film-forming solution in a proportion ranging from 5 to 66% (grams per 100 g of dry protein) followed by 20 min of stirring. It should be noted that gliadin films could not be formed at glycerol concentrations >33% at 50% relative humidity (RH) or >22% at 75% RH. Measured volumes of the film-forming solutions were poured onto a horizontal flat poly(tetrafluoroethylene) (PTFE) tray, and water and ethanol were allowed to evaporate. Films were dried at 23 ± 2 °C and 50 ± 5% RH for 10 h and peeled off the casting surface.

Film Thickness. Film thickness was measured using 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.

Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS-PAGE). Cysteine-treated material and control gliadin-rich material extracted from wheat gluten were analyzed by SDS-PAGE performed in a vertical electrophoresis unit (Hoefer Scientific Instrument, San Francisco, CA). The procedure used was that of Laemmli (*15*) with some minor modifications based on the procedure of Ng and Bushuk (*16*). One hundred microliters of film-forming solution from control or cysteine-treated gliadins was mixed with 100 μL of SDS sample buffer [0.063 M Tris-HCl, pH 6.8, 2% w/v SDS, 0.01% (w/v) Pyronin as a gel marker dye] with or without reduction of disulfide bonds with 5% (v/v) 2-mercaptoethanol. Each sample/buffer mixture was allowed to stand at room temperature for 2 h with occasional vortexing and finally centrifuged at 13800*g* for 10 min. Ten microliters of the clear top layer of each sample was loaded into each slot in the gel. After electrophoresis, the gel was stained with Coomassie Brilliant Blue G-250. The molecular weights of the protein standard mixture ranged from 36 kDa (glyceraldehyde-3-phosphate dehydrogenase) to 205 kDa (myosin) and were obtained from the Sigma Chemical Co.

Differential Scanning Calorimetry (DSC). Measurements of the glass transition temperature (T_g) of films without glycerol were performed using a differential scanning calorimeter (TA DSC 2920, TA Instruments Inc., New Castle, DE) equipped with a Universal V2.6B TA integrator. The instrument was calibrated using indium as a standard. Films were conditioned over P_2O_5 at 23 °C for 3 weeks before testing. Dry samples of 8 mg were placed in a hermetically sealed aluminum pan and heated from 23 to 190 °C at 5 °C/min under a nitrogen flow (50 mL/min). It is known that wheat gluten presents an endothermic relaxation peak superimposed on the glass transition (*17*). As T_g is a reversible phenomenon, a preliminary scan was performed to eliminate this relaxation, and a second scanning was run after rapid cooling to reveal the T_g value. T_g was recorded as the midpoint temperature of the shift in the baseline due to the change in heat capacity upon glass transition.

Weight Loss (WL) of Films after Immersion in Water. Film specimens were dried in a desiccator containing dry calcium sulfate and weighed to obtain the initial dry weight. Dry film samples of ∼500 mg were immersed in beakers containing 50 mL of distilled water at 23 °C for 24 h with periodical gentle manual agitation. Films were removed from the water and placed back in the desiccator until they reached a constant weight. Films were reweighed to obtain the final dry weight. The percentage weight loss in water (%WL) of the films was calculated using the following equation:

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

WL tests for each type of film were replicated three times.

Equilibrium Moisture Content at 50% RH. Film samples of ∼500 mg were previously conditioned in an environmental chamber at 50 ± 5% RH at 23 °C for at least 2 weeks. Once constant weight was obtained, the equilibrium moisture content was determined by drying the samples in a vacuum oven at 60 °C for 24 h according to the method described by Karmas (*18*). Moisture content was calculated on a dry basis and reported as the average of three samples from different batches of films.

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 (EB) of films according to ASTM standard D-882-91 (*19*). Sample films were cut into 2.54 cm wide strips at least 10 cm long. The Instron grip separation was set at 5.08 cm and the cross-head speed at 50.8 cm/min. TS and EB values were reported in pascal and percent of length increase divided by original grip separation, respectively. Films were preconditioned at 23 ± 2 °C and 50, 75, or 90 ± 5% RH in environmental chambers for 72 h prior to testing. At least 10 samples of each type of film were measured.

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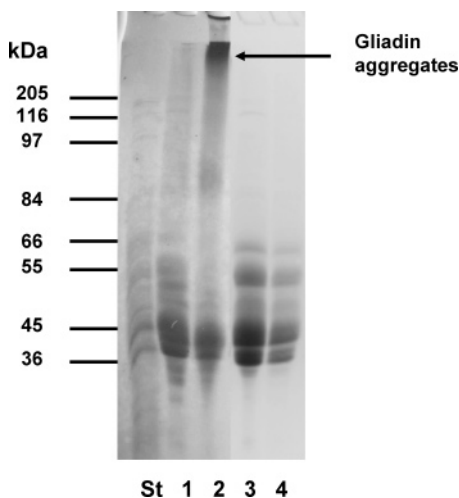
Figure 1. SDS-PAGE in the absence (lanes 1 and 2) or presence (lanes 3 and 4) of 2-mercoaptoethanol: lane 1, gliadins extracted from wheat gluten; lane 2, gliadins extracted from wheat gluten cross-linked with cysteine; lanes 3 and 4, gliadins and gliadins reacted with cysteine, respectively, under reducing conditions. St, molecular standard, myosin, 205 kDa; β-galactosidase, 116 kDa; phosphorylase *b*, 97 kDa; fructose-6-phosphate kinase, 84 kDa; albumin, 66 kDa; glutamic dehydrogenase, 55 kDa; ovalbumin, 45 kDa; glyceraldehyde-3-phosphate dehydrogenase 36 kDa.

Statistical Analysis. Statistical analysis of the results was performed using SPSS commercial software (SPSS Inc., Chicago, IL). When the ANOVA test indicated a significant ($p < 0.05$) difference among means, a least significant difference test was used to identify which film means differed significantly. The data were analyzed and graphically plotted using Sigma-plot software (Systat Software Inc., Richmond, CA).

RESULTS AND DISCUSSION

SDS-PAGE Analysis. SDS-PAGE analysis shown in **Figure 1** was conducted to investigate the polymerization of gliadins via the formation of intermolecular disulfide bonds. Lane 1 shows gliadin ethanol solution, and lane 2 shows gliadin ethanol solution after reaction with cysteine. Lanes 3 and 4 correspond to lanes 1 and 2, respectively, after the gliadin solution was treated with 2-mercaptoethanol in the buffer system. In lane 2, the formation of protein aggregates at the top of the separation gel is apparent. These aggregates, however, are absent in gliadin solution (lane 1). This indicates that cysteine is promoting gliadin polymerization. Upon cleavage of disulfide bonds with 2-mercaptoethanol these aggregates disappeared (lane 4), thus supporting the contention that cysteine treatment results in intermolecular cross-linking of gliadins and the formation of high molecular weight protein aggregates. Furthermore, and consistent with the formation of only disulfide covalent bonds in gliadins cross-linked with cysteine, no additional bands appeared in lane 4 compared to lane 3.

DSC. Because gluten proteins are amorphous polymers, they are characterized by a temperature-dependent equilibrium between a glassy and a rubbery state. The temperature at which phase transition occurs is governed by the chemical nature of the polymer, the presence or absence of plasticizer, and physical characteristics of molecules such as molar mass, branching, and cross-linking. It has been established that intermolecular covalent bonds as well as non-covalent interactions elevate the glass transition temperature of most synthetic polymers (*20, 21*). **Figure 2** shows the second DSC scan obtained for unplasticized dry films made from cysteine-treated gliadins, gliadins, or glutenins. The T_g values of films were within the range given

by several authors for wheat gluten proteins (*17, 22−27*) and are comparable with values reported for gelatin and soy protein-based films (*28, 29*). Gliadin films presented slightly lower T_g values than those found for glutenin films as observed by Micard et al. (*17*) using modulated DSC. Cocero and Kokini (*30*) and deGraff et al. (*31*) also reported that gliadins had a lower T_g than gluten and glutenin when determined by DSC and mechanical spectrometry, which was attributed to their lower molecular weight and lower concentration of hydrophilic amino acids. The formation of intermolecular disulfide bonds gave rise to an appreciable increase in the T_g of cross-linked gliadins, which could be explained by the decrease in the mobility of polypeptide chains within the film network. This increase in T_g in line with cross-linking has also been reported for other proteins and polysaccharides, such as formaldehyde cross-linked whey proteins, glutaric aldehyde cross-linked gelatin, trans-glutaminase cross-linked casein, and oxidatively cross-linked American corn hemicellulose (*32−35*).

Weight Loss in Water. Although the presence of nonpolar amino acid side groups contributes to the lack of solubility of gluten proteins in aqueous solution, gluten protein insolubility arises predominantly from the lack of ionizable groups and the very high molecular weight of the glutenins (*36*). Although solubility of films in water may be required for specific applications such as edible coatings and in the packaging of food ingredients for food processing, water resistance of films is an important issue in food packaging. Despite the fact that most of the films studied did not dissolve in water, they did suffer some weight loss owing to the diffusion of glycerol into the water. The loss of weight in water of films formed from gliadins was not evaluated because these films broke up, showing that non-covalent intermolecular forces between glia-dins could not maintain their integrity. When gliadins were treated with cysteine, the formation of intermolecular disulfide bonds gave rise to the formation of a more compact protein network that did not disintegrate upon immersion in water. Films made from other proteins presenting greater solubility in water than gluten proteins, such as soy protein isolate, sodium caseinate, cottonseed protein, and egg white protein, also showed a decrease in their solubility in water when chemical or physical cross-linking treatment was applied (*37−41*).

Loss of weight in films formed from glutenin and cysteine-treated gliadin was evaluated as a function of glycerol content and is summarized in **Table 1**. As can be observed, weight loss increased in concert with glycerol content, following linear models:

$$\%WL_{glutenin\ films} = 0.95 \pm 0.03 \times [\%\ glycerol] + 5.4 \pm 0.82, \quad R^2 = 0.998 \quad (2)$$

$$\%WL_{gliadin+cy-SH\ films} = 0.91 \pm 0.03 \times [\%\ glycerol] + 6.5 \pm 0.6, \quad R^2 = 0.997 \quad (3)$$

These results show that the mass lost in water practically corresponded to the films' glycerol content. After testing, films were found to be extremely brittle, a characteristic to be expected as a consequence of the loss of glycerol during water immersion.

Equilibrium Moisture Content (EMC) of Films at 50% RH. The EMC of films increased linearly with the increase in glycerol concentration in both gliadin and cysteine-treated gliadin films as can be observed in **Figure 3**. Glycerol is very hygroscopic and tends to draw water molecules into the protein film. This linear behavior has also been reported for whey protein isolate films containing glycerol (*42*) and for β-lacto-
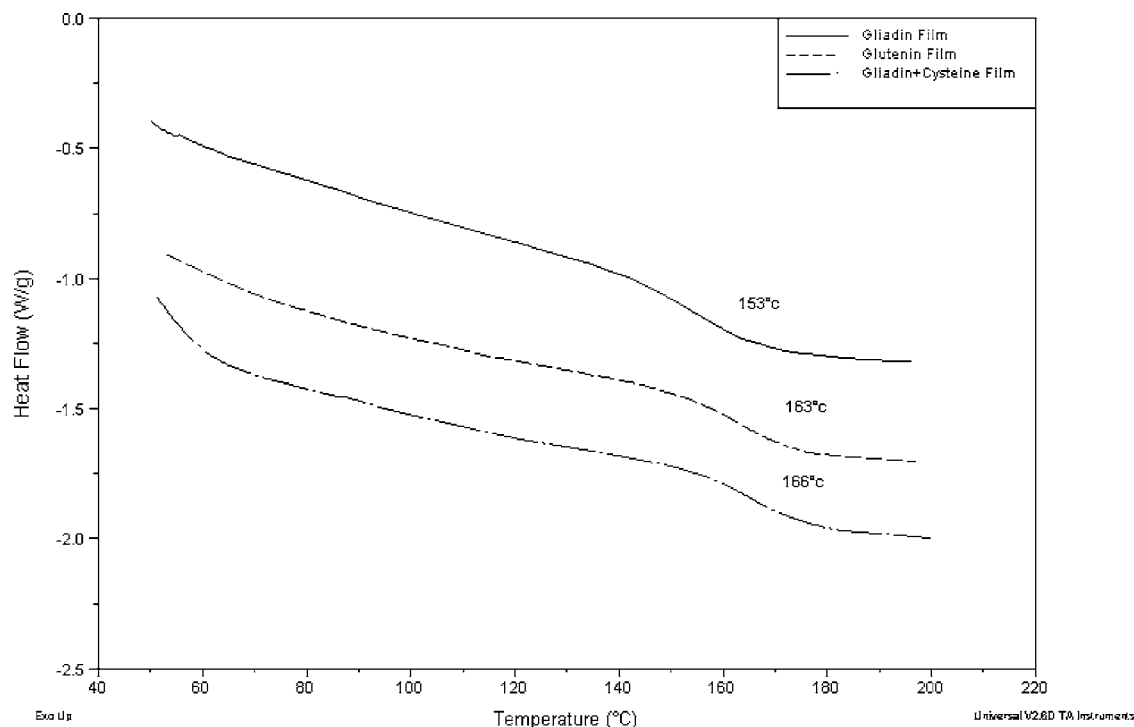
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Figure 2. DSC thermograms of unplasticized films made from gliadins, gliadins treated with cysteine, or glutenins.

Table 1. Weight Loss of Protein Films in Water Measured at 23 °C

	wt loss in water[a]		
% glycerol[b]	gliadin	gliadin + CYS	glutenin
0	D[c]	6.4 ± 1.0	6.6 ± 1.3
11	D	15.8 ± 2.0	16.0 ± 1.0
22	D	21.8 ± 0.7	22.0 ± 2.1
33	D	29.6 ± 3.6	30.5 ± 0.1
44	D	34.6 ± 2.1	34.2 ± 0.3
55	D	38.5 ± 0.8	38.4 ± 1.5
66	D	44.1 ± 1.5	43.3 ± 1.9

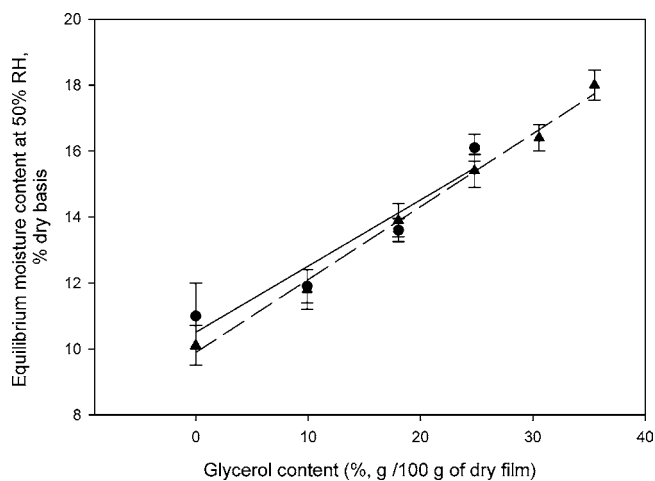[a] Grams per 100 g of dry film. [b] Grams of glycerol per 100 g of dry protein. [c] Disintegrated.

Figure 3. Effect of glycerol on the equilibrium moisture content of films made from cysteine-treated and untreated gliadins extracted from wheat gluten measured at 50% RH and 23 °C.

globulin and whey protein films plasticized with sorbitol (*43*). Gliadin films could not be measured at glycerol concentrations <33% (grams per 100 g of dry protein) because the resulting films were very sticky and could not be handled. In contrast,

films made from polymerized gliadins could retain large amounts of glycerol owing to the development of a more reticulated network. The dependency of the EMC of cysteine cross-linked and native gliadin films on glycerol can be described by the following linear regression equations:

$$\text{EMC}_{\text{gliadin films}} =$$
$$0.20 \pm 0.04 \times [\% \text{ glycerol}] + 10.5 \pm 0.7, \quad R^2 = 0.917 \quad (4)$$

$$\text{EMC}_{\text{gliadin+cy-SH films}} =$$
$$0.22 \pm 0.02 \times [\% \text{ glycerol}] + 9.8 \pm 0.2, \quad R^2 = 0.994 \quad (5)$$

Amino acid composition, the number of exposed polar groups, conformation, and surface polarity are all determining factors in the moisture sorption capacity of proteins. Previous studies have indicated the importance of hydrophilic groups in the binding of water molecules through hydrogen bond formation (*44−46*). Polymerization of gliadins via the formation of intermolecular disulfide bonds did not modify the moisture-holding capacity of the films. The similarity in amino acid composition between gliadins and glutenins could explain their similar water sorption behaviors.
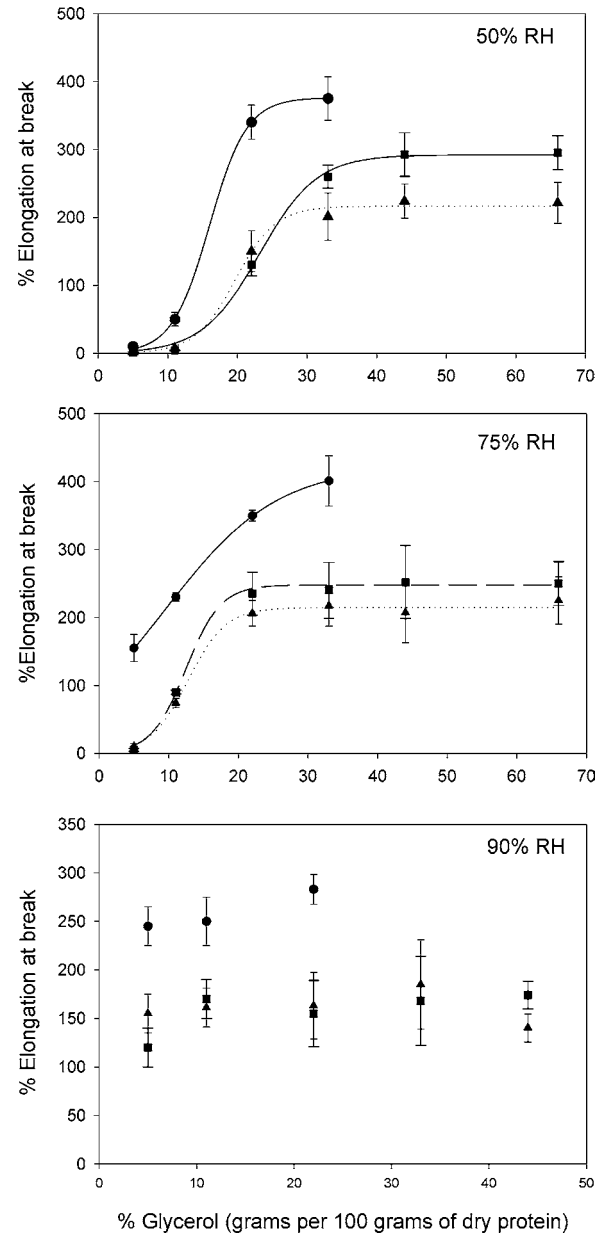
Mechanical Properties. It is well-known that plasticizers such as water and glycerol have a considerable influence on the mechanical properties of hydrophilic biopolymers. Plasticizers act by disrupting chain−chain secondary forces and increasing the free volume of the material resulting in the formation of a loose polymer matrix. **Figures 4** and **5** show the effects of glycerol content and RH on the tensile strength (TS) and elongation to break (EB) of glutenin, gliadin, and cysteine-treated gliadin films. TS decreased as the glycerol content in films increased for samples tested at 50 and 75% RH, although the decrease in TS was more acute for samples tested at 50% RH because water acts as a strong plasticizer, thus masking the effect of glycerol. The extensibility of the films increased with the glycerol content until a plateau was reached at glycerol concentrations above 33 and 22% for relative humidities of 50 and 75%, respectively. At 90% RH the mechanical properties

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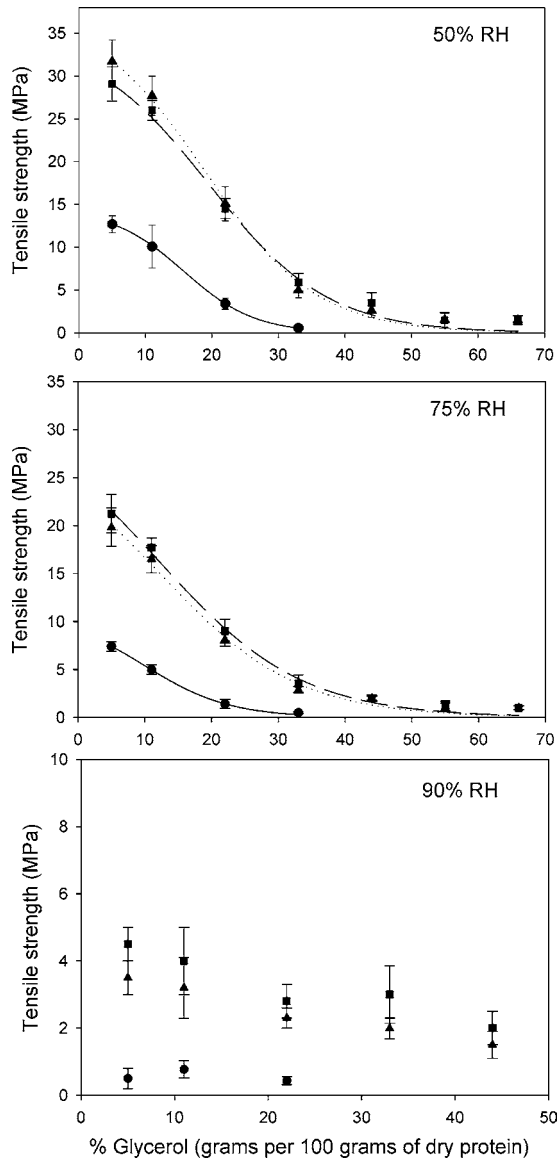
Figure 4. Effect of glycerol on the tensile strength of films made from gliadins, gliadins treated with cysteine, or glutenins measured at 23 °C and 50, 75, or 90% RH: gliadin films (●, experimental values; —, modeled curve); cysteine-treated gliadin films (▲, experimental values; · · ·, modeled curve); glutenin films (■, experimental values; - - -, modeled curve).

Figure 5. Effect of glycerol on the elongation at break for films made from gliadins, gliadins treated with cysteine, or glutenins measured at 23 °C and 50, 75, or 90% RH: gliadin films (●, experimental values; —, modeled curve); cysteine-treated gliadin films (▲, experimental values; · · ·, modeled curve); glutenin films (■, experimental values; - - -, modeled curve).

of films varied very little with changes in glycerol content, thus indicating that the films were completely plasticized by water.

TS values for films obtained from cysteine-mediated polymerized gliadins were similar to those obtained for films made from glutenins across the range of glycerol concentrations and relative humidities studied, and no significant differences were found between them ($P > 0.05$). There were significant differences between the TS values of these films and those of gliadin films ($P < 0.05$). These findings are consistent with the development of intermolecular disulfide bonds between polypeptide chains, leading to increased strength of the protein network compared to untreated gliadin films. In gliadin films, intermolecular interactions are mediated by secondary forces, principally the formation of hydrogen-bonded β-sheet aggregates (*47*). The extensibility of gliadin films decreased after treatment of proteins with cysteine, these differences being significant ($P < 0.05$) for glycerol concentrations >5% when measurements where done at 50% RH. At 75 and 90% RH these differences were significant ($P < 0.05$) across the whole range of glycerol

concentrations tested. This behavior is probably due to a greater density of intermolecular disulfide bond cross-linked gliadins, thus resulting in a stiffer polymer network. Cross-linked gliadins films have lower EB values compared to glutenin films for plasticizing glycerol concentrations >22% measured at 50% RH, and >11% for films measured at 75% RH ($P < 0.05$). No significant differences were found for EB of cross-linked gliadin and glutenin films measured at 90% RH except when the plasticizing concentration was 44% ($P < 0.05$).

The tensile stress−strain behavior of films can be classified into two types according to **Figure 6**. The type I curve corresponds to the mechanical behavior of gliadin films characterized by a low elastic modulus and a high EB, which is in agreement with the viscous nature of gliadins. The type II curve describes the mechanical behavior corresponding to films made from glutenins or cysteine-treated gliadins and reflects a

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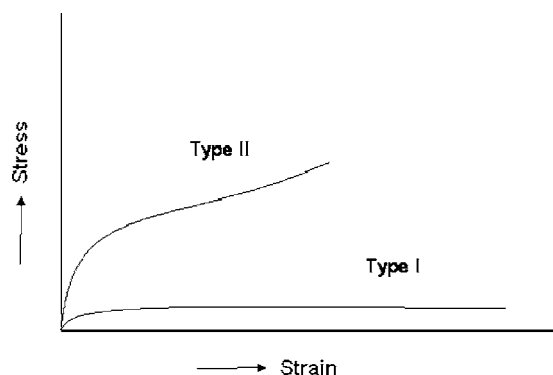
Figure 6. Stress–strain curves for films measured at 23 °C, 50% RH, and 33% glycerol content

Table 2. Constants for Tensile Strength (TS) versus Glycerol Content Curves of Films Made from Gliadins, Gliadins Treated with Cysteine, or Glutenins Measured at 23 °C and 50 or 75% Relative Humidity

film type	TS_0	X_c	a	R^2
	50% Relative Humidity			
gliadin	14.4	15.8	5.4	0.955
gliadin + CYS	37.5	19.1	8.3	0.978
glutenin	34.3	20.1	8.8	0.994
	75% Relative Humidity			
gliadin	10.9	9.8	6.4	0.987
gliadin + CYS	32.3	12.4	10.6	0.942
glutenin	29.8	12.4	10.2	0.965

stronger, stiffer, and less extensible protein network. In this regard, small deformation oscillatory measurements made with gliadin showed a large increase in the elastic component when they were treated at temperatures >70 °C attributed to the formation of a network structure through cross-linking reactions (*48*).

The sigmoidal relationship between the mechanical properties of films and their glycerol content at constant ambient temperature can be described by Peleg's model (*49−52*)

$$Y(X) = Y_0/\{1 + \exp[(X - X_c)/a]\} \qquad (6)$$

where Y is the calculated value of the mechanical parameter at plasticizer concentration X, Y_0 is the maximum value of this parameter, X_c is the concentration of plasticizer that decreases Y to a value that is 50% of Y_0, indicating the loss of mechanical integrity, and a is a dimensionless constant and accounts for the steepness of the drop in the magnitude of Y. Thus, when a tends to zero, the shape of the curve approaches a step function, whereas for higher values of a the curve is rather flat. This is an empirical model but it can be used to characterize and compare different materials. This model has recently been fitted to sodium caseinate and to chitosan films blended with pullulan or starch (*53−55*). The parameters obtained from fitting the model to diverse films and storage conditions of 50 or 75% RH at 23 °C are given in **Tables 2** and **3**, respectively. There is good agreement between the experimental data and the theoretical curves plotted in **Figures 4** and **5**. The TS_0 parameter, which predicts tensile strength for unplasticized films, presented higher values for glutenin and cysteine-treated gliadin films than for gliadin films (**Table 2**) exposed to either 50 or 75% relative humidities. TS_0 values decreased as RH increased owing to the gain of water by proteins and consequent plasticization of the film matrix. From the values of parameter X_c obtained under 50 or 75% RH conditions (see **Table 2**) it can be seen that films containing interchain covalent bonds in their structure

Table 3. Constants Obtained for Elongation at Break (EB) versus Glycerol Content Curves of Films Made from Gliadins, Gliadins Treated with Cysteine, or Glutenins Measured at 23 °C and 50 or 75% Relative Humidity

film type	E_0	X_c	a	R^2
	50% Relative Humidity			
gliadin	374	15.9	2.6	0.997
gliadin + CYS	216	19.8	2.9	0.992
glutenin	307	21.3	3.1	0.944
	75% Relative Humidity			
gliadin	421	9.5	7.8	0.999
gliadin + CYS	215	12.9	2.9	0.996
glutenin	248	12.5	2.5	0.998

required a higher glycerol content for TS_0 to drop to 50%. X_c values obtained from tensile strength curves correlate well with those from elongation at break curves (compare **Tables 2** and **3**). It is noteworthy that the drop in mechanical properties noted for films kept at 50 and 75% RH was not observed for films kept at 90% RH. This may be explained on the basis of the changes that polymers undergo around their glass transition temperature. According to previous studies into the effect of water content on the glass transition temperature of wheat gluten films (*17, 27*), films without glycerol conditioned at 23 °C and at 50 or 75% RH were below their T_g. The T_g value decreased when glycerol was added and continued to decrease as the glycerol content in the film increased. The curves plotted in **Figures 4** and **5** at 50 and 75% RH reflect the drop in T_g as the glycerol content increases, with a leveling off at high glycerol content. However, at 23 °C and 90% RH, films are highly plasticized by water and well above their T_g, which explains why little effect of glycerol content was observed.

Conclusions. From the above results it can be concluded that cysteine is effective in promoting the cross-linking of gliadins through the formation of interchain disulfide bonds. The resulting films are water resistant and also harder and less extensible than films made from unmodified gliadins. Their mechanical properties are similar to those of films made from glutenins, but cross-linked proteins are soluble in 70% ethanol, whereas gluten or glutenins are not. Cysteine offers a promising alternative to other harmful cross-linkers, such as aldehydes, in order to process proteins into bioderived films for diverse applications such as food packaging or coatings.

ABBREVIATIONS USED

Cys-SH, cysteine; D, disintegrate; EB, elongation at break; EMC, equilibrium moisture content; RH, relative humidity; T_g, glass transition temperature; TS, tensile strength; WL, weight loss.

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