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Development and Characterization of Biodegradable Films Made from Wheat Gluten Protein Fractions†

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Gliadins and glutenins were extracted from commercial wheat gluten on the basis of their extractability in ethanol and used to produce film-forming solutions. Films cast using these gliadin- and glutenin-rich solutions were characterized. Glycerol was used as a plasticizer, and its effect on the films was also studied. Films obtained from the glutenin fraction presented higher tensile strength values and lower elongation at break and water vapor permeability values than gliadin films. Gliadin films disintegrated when immersed in water. The GAB isotherm model was used to describe the equilibrium moisture sorption of the films. The glycerol concentration largely modified mechanical and water vapor barrier properties of both film types.

KEYWORDS: Edible films; gliadins; glutenins; tensile strength; water vapor permeability

INTRODUCTION

Wheat gluten is composed of a mixture of complex protein molecules that can be separated into glutenins and gliadins on the basis of their extractability in aqueous ethanol (*1*). Neither of these groups consists of pure proteins, and a considerable amount of overlap may occur depending on the extraction conditions (*2*).

Gliadin provides the viscous component of gluten and constitutes a heterogeneous group of proteins characterized by single polypeptide chains associated by hydrogen bonding and hydrophobic interactions, having intramolecular disulfide bonds, and being soluble in a 70% ethanol/water solution. These proteins have been classified into four groups: α-, *β*-, and *γ*-gliadins of relative molecular weight masses (M_r) between 30000 and 50000 by sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS-PAGE) and *ω*-gliadins with M_r of about 44000−74000 by SDS-PAGE (*3*).

Glutenins form an extensive network of intermolecular disulfide bonds. As with synthetic polymers (*4*), the larger the subunits involved in the cross-linking the greater the contribution to the elastic properties of the gluten matrix. Glutenins comprise a diverse number of protein molecules grouped into high molecular weight subunits with M_r in the range of 95000−145000 by SDS-PAGE (*5*) and low molecular weight subunits with M_r around 44000 by SDS-PAGE (*6*). Hydrogen bonding between the repeat regions of high molecular weight proteins has been found to be responsible for the elasticity of gluten according to the "loop and train" theory of Belton (*7*).

Low molecular weight glutenins are partially soluble in 70% ethanol and are analogous with some high molecular weight gliadins (*8*). Gliadins and glutenins are present in almost equal quantities in wheat gluten and have similar amino acid compositions, being high in both glutamine and proline. They also have a considerable number of nonpolar amino acids containing aliphatic or aromatic groups. These groups, together with a few readily ionizable amino acids, are responsible for the insolubility of gluten in water (*9*). The amount, size distribution, and molecular architecture of glutenins and gliadins greatly influence the rheological, processing, mechanical, and physicochemical properties of gluten (*10*).

An alternative classification for wheat gluten proteins has been proposed by Shewry et al. (*11*) on the basis of the biological, chemical, and genetic relationships among them. These authors divide wheat gluten proteins into three groups: high molecular weight prolamins (HMW subunits of glutenin), the S-poor prolamins (*ω*-gliadins), and the S-rich prolamins. The latter group includes gliadins (α, *β*, and *γ*) and low molecular weight glutenins.

Wheat gluten is a renewable material widely used in bakery products as part of flour (*12*). However, one of the major problems of gluten for the food industry is its poor solubility in water, which limits its applications (*13*−*15*). Currently, there is a need to find new uses for gluten through the development of nonconventional applications such as the manufacture of films that could be used in food packaging provided they are strong and relatively transparent. Similarly to polyamides (*16*), wheat

† This work is dedicated to the memory of Prof. Rubén Hernández.

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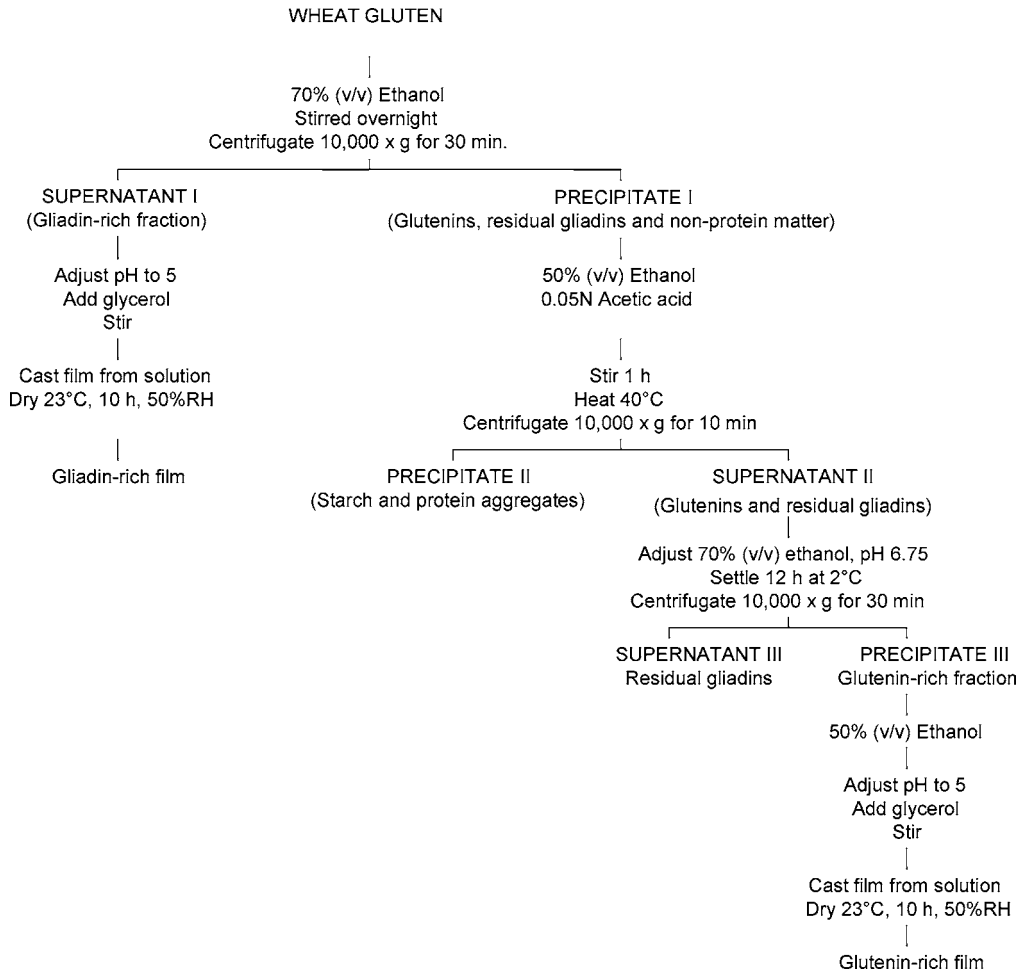

Figure 1. Flow diagram of the preparation of gliadin- and glutenin-rich fractions and production of their respective films.

gluten films are good oxygen barriers at low relative humidity. However, wheat gluten films have poor water barrier properties. Addition of a plasticizer, such as glycerol, is required to impart flexibility and extensibility to the films. Methods to produce edible and biodegradable films from wheat gluten have been reported by several authors (*17*−*23*). However, little is found in the literature regarding the separation of gluten into its main components, gliadins and glutenins, as purer sources for films.

The aim of this study was to develop a procedure to produce films from the gliadin-rich and glutenin-rich fractions of wheat gluten and evaluate the suitability of these films for food packaging with respect to their mechanical, optical, and water vapor barrier properties.

MATERIALS AND METHODS

Reagents. Crude wheat gluten, glycerol, ethanol, acetic acid, Tris, Coomassie Brilliant Blue G-250, and primary standard reference proteins were obtained from Sigma Chemical Co. (St. Louis, MO). Electrophoretic grade acrylamide, bisacrylamide, and sodium dodecyl sulfate (SDS) were obtained from Bi-Rad (Richmond, CA). All chemicals used were analytical grade reagents.

Extraction of Gliadin- and Glutenin-Rich Fractions. A schematic diagram of the extraction method is presented in **Figure 1**. Initially, 100 g of crude wheat gluten was dispersed in 400 mL of 70% (v/v) ethanol/water, stirred overnight at room temperature, and centrifuged at 10000*g* for 30 min at 23 °C. The resulting supernatant (supernatant I), 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, yielding supernantant II. Glutenins were separated from residual gliadins remaining in supernatant II by precipitation with ethanol. Ethanol was added to supernatant II to a final concentration of 70% (v/v). The pH was adjusted to 6.75 with sodium hydroxide and the mixture left for 12 h at 2 °C. The precipitated glutenins were obtained by centrifugation at 10000*g* for 30 min and dispersed in 50% (v/v) ethanol/water.

The protein contents of the gliadin- and glutenin-rich fractions were 34 and 18.5% (grams/100 g of gluten), respectively, and were determined using micro-Kjeldahl method 46-13 (*24*) after previous evaporation of the solvent.

Film Formation and Conditioning. The protein contents of the gliadin- and glutenin-rich fractions were adjusted to 7.5% (grams of protein to 100 g of solution). The pH of each fraction was brought to 5 with acetic acid. Glycerol was then added as a plasticizer at concentrations ranging from 11 to 66% (grams per gram of dry protein) and stirred for 20 min. Measured volumes of the film-forming solution were poured onto a horizontal flat poly(tetrafluoroethylene) tray to allow water and ethanol to evaporate. The films were dried at 23 ± 2 °C and 50 ± 5% relative humidity (RH) for 10 h. Dried films were peeled off the casting surface and preconditioned in a chamber at 23 ± 2 °C and 50 ± 5% RH for at least 72 h prior to testing their optical, mechanical, and water barrier properties.

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.

Sodium Dodecyl Sulfate—Polyacrylamide Gel Electrophoresis. The gliadin- and glutenin-rich fractions were analyzed by SDS-PAGE electrophoresis performed in a vertical electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA). The procedure used was

that of Laemmli (*25*) with some minor modifications based on the procedure of Ng and Bushuk (*26*). Ten milligrams of ground gliadin or glutenin films without glycerol, 10 mg of commercial wheat gluten, or 100 μL of film-forming solution (gliadin- or glutenin-rich fraction without glycerol) was mixed with 200 μL of SDS sample−buffer [0.063 M Tris-HCl, pH 6.8, 2% (w/v) SDS with or without 5% (v/v) disulfide bond-cleaving 2-mercaptoethanol (2-ME), 0.01% (w/v) Pyronin]. Each sample−buffer mixture was allowed to stand at room temperature for 2 h with occasional shaking and 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 standard protein mixture ranged from 205 kDa (myosin) to 36 kDa (glyceraldehyde-3-phosphate dehydrogenase) and were obtained from Sigma Chemical Co.

Film Color. Film color was measured with a CR-300 Chroma meter (Minolta Camera Co., Ltd., Osaka, Japan). Film specimens were placed on a white standard plate, and the results were given in CIE $L^*a^*b^*$ using a D65 illuminant/10° observer. L^* (lightness), a^* (red component), and b^* (yellow component) were measured. Samples were measured in triplicate. Five measurements at different locations were obtained from each film sample.

Opacity. Film apparent opacity was evaluated according to a modified standard procedure as described by Gontard et al. (*21*). A Perkin-Elmer Lambda 25 UV−vis spectrophotometer (Perkin-Elmer Instruments, Shelton, CT) was used to evaluate the absorbance spectrum of each film at a range of 400−800 nm. Apparent opacity was defined as the integrated area under the curve, calculated using UV-WIN-Lab software and expressed as an absorbance value (*A*) wavelength (nm) product (*A*·nm). Samples were measured in triplicate.

Loss of Weight of Films after Immersion in Water at 23 °C. Films were dried in a desiccator containing dry calcium sulfate. Preweighed dry film samples of ~500 mg were immersed in beakers containing 50 mL of distilled water at 23 °C for 24 h with periodic gentle manual agitation. Films were removed from the water and placed back in the desiccator until they reached a constant weight, which was used as the final dry weight of the film. The weight loss percentage (% WL) of each film sample was calculated using the following equation:

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

Tests for each type of film were repeated three times.

Water Vapor Permeability (WVP). The water vapor transmission rate [g/(m²·s)] through films was measured using a Permatran W3/30 (MoCon Inc., Minneapolis, MN) at 10, 23, 30, and 37 °C with a RH gradient of 50 to 0% (in dry nitrogen) across the film. At least three samples of each type of film produced were measured. Permeability values are reported as water permeability coefficient in [(g·m)/(m²·s·Pa)].

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 (PE) of films according to ASTM standard D-882 (*27*). Sample films were cut into 2.54 cm wide strips of at least 10 cm length. The Instron grip separation was set at 50.8 mm and the cross-head speed at 508 mm/min. TS and PE values were reported in Pascals and in percent of length increase divided by the original grip separation, respectively. At least 10 samples from each type of film were evaluated.

Moisture Sorption Isotherms. Films were cut into small pieces weighing 4−5 g, placed in aluminum dishes, and allowed to reach the corresponding moisture equilibrium when exposed to various relative humidities created by eight different salt solutions kept in 10 L sealed containers placed in an environmental chamber conditioned at 25 °C. The relative humidity values in the containers were 11.3 ± 0.3, 22.5 ± 0.3, 32.8 ± 0.2, 43.2 ± 0.4, 52.9 ± 0.2, 0.66 ± 0.05, 75.3 ± 0.1, and 93.6 ± 0.2, obtained using saturated salt solutions of $LiCl \cdot H_2O$, $KC_2H_3O_2$, $MgCl_2 \cdot 6H_2O$, $K_2CO_3 \cdot 2H_2O$, $Mg(NO_3)_2 \cdot 6H_2O$, $NaNO_2$, NaCl, and KNO_3, respectively (*28*). Once constant weight was obtained (after ~2 weeks), the samples were removed and dried in a vacuum oven at 60 °C for 24 h (*29*). The equilibrium moisture content was calculated on a dry basis and reported as the average of three replicates from different batches at each RH condition. The experimental values were fitted by the Guggenheim−Anderson−deBöer (GAB) model

$$\text{EMC} = W_m C k a_w / [(1 - k a_w)(1 - k a_w + C k a_w)] \quad (2)$$

where EMC = equilibrium moisture content on a dry basis, W_m = Brunauer−Emmett−Teller (BET) monolayer moisture content and represents the water content corresponding to 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 constants W_m, k, and C were calculated according to the method of Bizot (*30*). To evaluate the goodness of the fit, the mean relative percentage deviation modulus (E) was calculated as

$$E\,(\%) = \frac{100}{N} \sum \frac{|M^{\text{exptl}} - M^{\text{calcd}}|}{M^{\text{exptl}}} \quad (3)$$

where N is the number of experimental points, M^{exptl} is the experimental equilibrium moisture content value, and M^{calcd} is the calculated equilibrium moisture content value. The lower the E value, the better the fit.

The clustering function developed by Zimm and Lundberg (*31*) has been used to calculate the self-association of water molecules in the polypeptide matrix. Their clustering function, G_{11}/V_1, is

$$\frac{G_{11}}{V_1} = -(1 - V_1)\frac{\partial(a_w/V_1)}{\partial a_w} - 1 \quad (4)$$

When G_{11}/V_1 is > -1, water is expected to cluster. V_1 is the volume fraction of the solute (water).

RESULTS AND DISCUSSION

General Properties. Unplasticized gliadin- and glutenin-rich films were not studied because they were too brittle to be handled for testing. Films plasticized with glycerol were flexible and homogeneous, with a yellowish color that can be attributed to the presence of chromophores in wheat gluten. Film thickness was ~40 ± 8 μm for all prepared samples. Gliadin-rich films made with glycerol concentrations >33% (grams per 100 g of dry protein) were sticky, difficult to handle, and not evaluated further. At glycerol concentrations of <11%, the resulting gliadin- and glutenin-rich films were very brittle.

SDS-PAGE. SDS-PAGE patterns of nonreduced and reduced samples of wheat gluten, films, and film-forming solutions of gliadin- and glutenin-rich fractions are shown in **Figure 2**. No differences were observed between the electrophoretic patterns of the nonreduced gliadin-rich fraction and the nonreduced gliadin-rich film, nor between those of the glutenin-rich fraction and the glutenin-rich films. Likewise, no SDS-PAGE pattern differences were noted between these fractions and film samples run under reduced conditions. These findings suggest that covalent bonds other than disulfide bonds were not involved in film formation. Only the nonreduced samples of wheat gluten, the gliadin-rich film-forming solution, and the gliadin-rich film (lanes 2, 3, and 5, respectively) showed clear bands, which corresponded to molecular mobilities in the region of the gliadins. Nonreduced samples (lanes 2−6) showed the tendency to form aggregates on the top of the gel. These aggregates were likely the result of the formation of disulfide bonds between subunits through sulfhydryl−disulfide interchange reactions. Disulfide bonds were cleaved by reduction with 2-ME, and the disaggregated proteins were able to enter the gel. Reduced samples of glutenin-rich film-forming solution and glutenin-rich film (lanes 9 and 11), as well as the control gluten sample (lane 7), presented well-defined bands corresponding to typical

Table 1. Color Coordinate Values (L^*, a^*, b^*), Opacity, and Weight Loss in Water (WL) of Gliadin- and Glutenin-Rich Films

	L^*	a^*	b^*	opacity (A, nm)	% WL (g/100 g of dry film)
gliadin-rich films	97.3 ± 0.7	−0.19 ± 0.07	4.5 ± 0.3	34 ± 3	disintegrated
glutenin-rich films	95.5 ± 0.2	−0.21 ± 0.04	6.8 ± 0.5	101 ± 10	30.5 ± 0.1

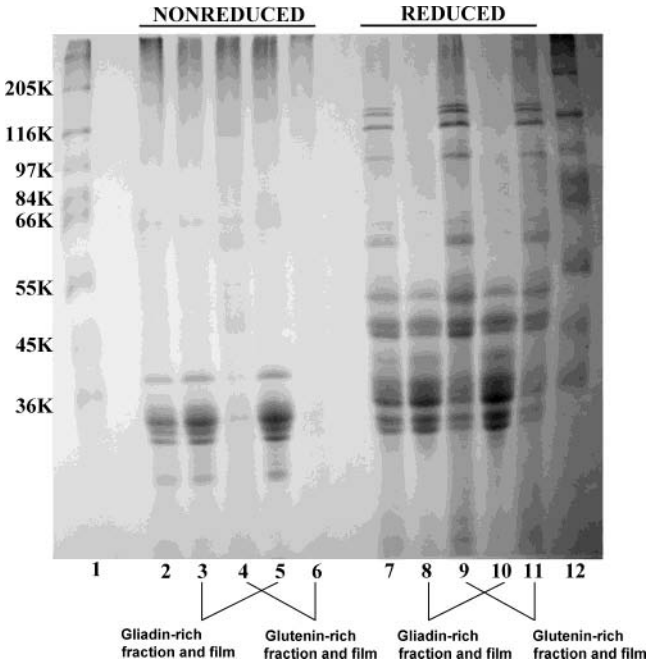

Figure 2. SDS-PAGE patterns of nonreduced (lanes 2–6) and 2-ME-reduced (lanes 7–11) samples: molecular markers (lanes 1 and 12), native wheat gluten (lanes 2 and 7), gliadin-rich fractions (lanes 3 and 8), glutenin-rich fractions (lanes 4 and 9), gliadin-rich films (lanes 5 and 10), and glutenin-rich films (lanes 6 and 11).

glutenins. There was some contamination of the glutenin fraction with gliadins (within the 36 and 55 kDa regions) that remained in the glutenin fraction even after the extraction and purification steps with 70% (v/v) ethanol.

Optical Properties. The optical properties of gliadin-rich and glutenin-rich films are shown in **Table 1**. The opacity values of films prepared from gliadins and glutenins were 34 ± 3 and 101 ± 10 A·nm, respectively. Gliadin-rich films showed greater transparency than glutenin films. Glutenin-rich films were slightly more yellow and darker than gliadin-rich films, as shown by the lower L^* and greater b^* values in **Table 1**.

Loss of Weight of Films in Water. Loss of weight was evaluated for glutenin films with a glycerol content of 33%. Gliadin-rich films could not be tested because they disintegrated after immersion in water. The low molecular weights of gliadins and their lack of intermolecular disulfide bonds could be responsible for the loss of film integrity upon contact with water. In contrast, glutenin-rich films kept their integrity after 24 h of immersion. Glutenins consist of high molecular weight polymers cross-linked by disulfide bonds, making the film more resistant to water. The loss of weight after immersion of glutenin-rich films was 30.5 g/100 g of dry film (**Table 1**). This quantity closely corresponds to the glycerol added to the film (25 g/100 g of dry film) plus some starch or short-chain polypeptides that are soluble in water. After testing, films were observed to be extremely brittle, consistent with the loss of glycerol during immersion.

Water Vapor Permeability (WVP). *Effect of Glycerol Concentration.* The effect of glycerol content on the WVP of gliadin- and glutenin-rich films is shown in **Figure 3**. WVP

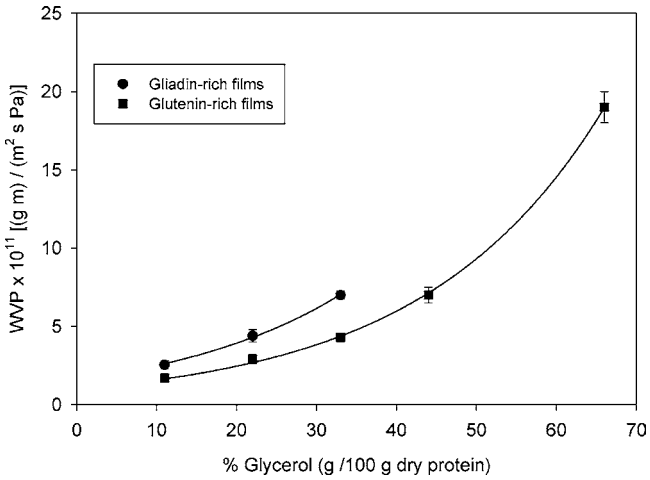

Figure 3. Effect of glycerol on water vapor permeability (WVP) of gliadin- and glutenin-rich films.

increased with increasing glycerol concentration from 2.54 × 10^{-11} to 7 × 10^{-11} [(g·m)/(m^2·s·Pa)] for gliadin-rich films and from 1.7 × 10^{-11} to 19 × 10^{-11} for glutenin-rich films across the range of glycerol concentrations studied, 11−66%. Although addition of glycerol enhances the flexibility of protein films, it reduces their water barrier properties (*32*). As **Figure 3** shows, the WVP of glutenin-rich films increases dramatically at plasticizer concentrations >40%, following an exponential relationship with increasing glycerol content. The incorporation of low molecular weight molecules such as glycerol into films increases the free volume of the polymer matrix and, consequently, the network becomes less dense, facilitating the diffusion of water molecules through the film. Besides the plasticizing effect of glycerol, this molecule possesses a high capacity to bond water molecules (*33*), and its presence in films increases their moisture sorption capacity.

Comparing the WVP values between gliadin- and glutenin-rich films, gliadin-rich films had slightly higher WVP values than glutenin-rich films at the plasticizer concentrations studied. These differences were greater at higher amounts of glycerol. Gliadins consist of low molecular weight monomeric units interconnected by hydrogen bonds and ionic and hydrophobic forces. In contrast to glutenins, there is a lack of intermolecular disulfide bonds in the gliadin structure. The longer molecular chains and the intermolecular disulfide bonds present in glutenins may restrict polypeptide chain mobility, and consequently, permeant mobility, diffusion rate, and permeability.

Temperature Effect. The WVP of gliadin- and glutenin-rich films plasticized with 33% glycerol (grams per 100 g of dry protein) was evaluated at 10, 23, 30, and 37 °C. WVP increased with temperature following the Arrhenius model equation

$$P = P_0 \exp(-E_p/RT) \quad (5)$$

where P_0 is a pre-exponential factor [g/(m·s·Pa)], E_p is the activation energy of permeability (kcal/mol), R is the universal gas constant (1.987 kcal/mol·K), and T is the absolute temperature (K). Plots of the natural logarithm of permeability versus reciprocal temperature were obtained for gliadin- and glutenin-

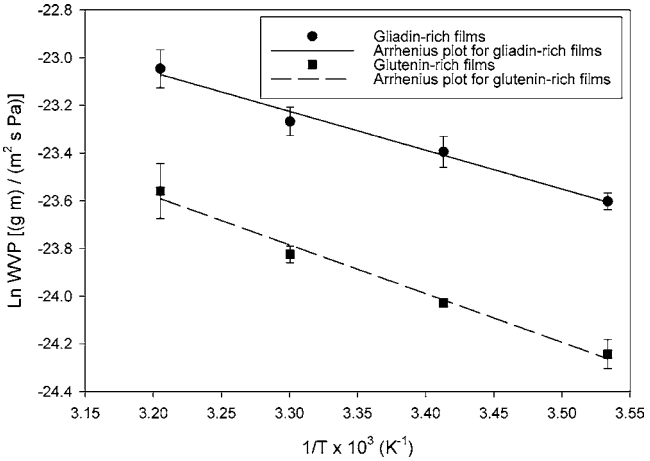

Figure 4. Arrhenius plots for water vapor permeability (WVP) of films made from gliadin- and glutenin-rich fractions (T = temperature).

Table 2. Activation Energies of Water Vapor Permeation (E_p) through Various Types of Films

reference	film	% RH[a]	E_p (kJ/mol)
present work	gliadin-rich	0–50	13.8 ± 1.3
present work	glutenin-rich	0–50	17.6 ± 1.3
Shogren, 1997 (*36*)	PHBV-6[b]	0–100	30
Shogren, 1997 (*36*)	PHBV-12[b]	0–100	30
Shogren, 1997 (*36*)	PHBV-18[b]	0–100	31
Shogren, 1997 (*36*)	PLA[c]	0–100	5
Shogren, 1997 (*36*)	PCL[d]	0–100	15
Shogren, 1997 (*36*)	aliphatic polyester	0–100	21
Shogren, 1997 (*36*)	poly(ester-amide)	0–100	11

[a] Percent relative humidity. [b] PHBV, poly(β-hydroxybutyrate-*co*-hydroxyvalerate); 6, 12, and 18% valerate. [c] PLA, poly(L-lactic acid); [d] PCL, poly(ε-caprolactone).

rich films and are shown in **Figure 4**. The fit of the model was assessed by the linearity of the curves ($R^2 = 0.984$ for gliadin-rich films and $R^2 = 0.988$ for glutenin-rich films). The transition step of a polymer from a glassy to a rubbery state affects the physical properties of the material, especially those governed by internal molecular mobility (*34*) such as permeability. No breaks were observed in the linear curves of the plots in the present study, indicating that there is no transition within the range of temperatures studied (10−37 °C).

Activation energy is "a measure of the energy expended against the cohesive forces of the polymer in forming the gaps through which diffusion occurs" (*35*). The activation energies of permeation (E_p) for gliadin- and glutenin-rich films were obtained from the slopes of plots of Ln P versus $1/T$ and are shown in **Table 2**. As can be observed, the values obtained for these films were in the same range as those found for other biodegradable polymers (*36*) (see **Table 2**). Glutenin-rich films showed higher E_p values than gliadin-rich films. Because the activation energy for permeation comprised the enthalpy of sorption of the permeant (ΔH_s) plus the activation energy of diffusion (E_d)

$$E_p = \Delta H_s + E_d \quad (6)$$

assuming E_d is the dominant term in the above equation, a higher activation energy of permeation in glutenin-rich films could imply a higher activation energy of diffusion compared to gliadin-rich films as a consequence of a tighter and more reticulated polymer matrix.

Moisture Sorption Isotherms. The water sorption isotherms of unplasticized and glycerol-plasticized gliadin- and glutenin-rich films measured at 23 °C are shown in **Figures 5** and **6**, respectively. The sorption isotherms are sigmoidal. The equilibrium moisture content of films increased slowly with increased water activities until water activities of about 0.55 or 0.65 depending on whether the films contain glycerol. Greater water activities implied a substantial water gain in the films due to the development of solvent−solvent interactions. Addition of glycerol to films increased their equilibrium moisture content as expected. The rise was more pronounced for plasticized films and occurred at lower water activity values than for films without glycerol. The moisture sorption capacities of gliadin- and glutenin-rich films were similar; this behavior was also observed for gliadin- and glutenin-rich films plasticized with glycerol and is attributable to the similar amino acid composition of these fractions.

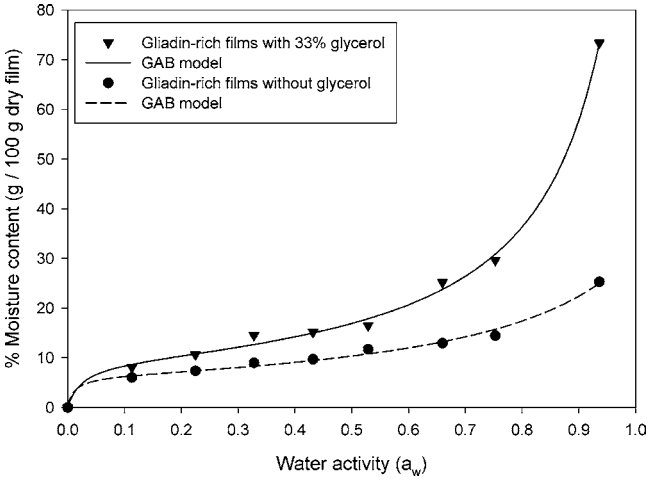

Figure 5. Experimental moisture sorption isotherms and curves predicted by the GAB model for gliadin-rich films plasticized with 0 and 33% glycerol at 23 °C.

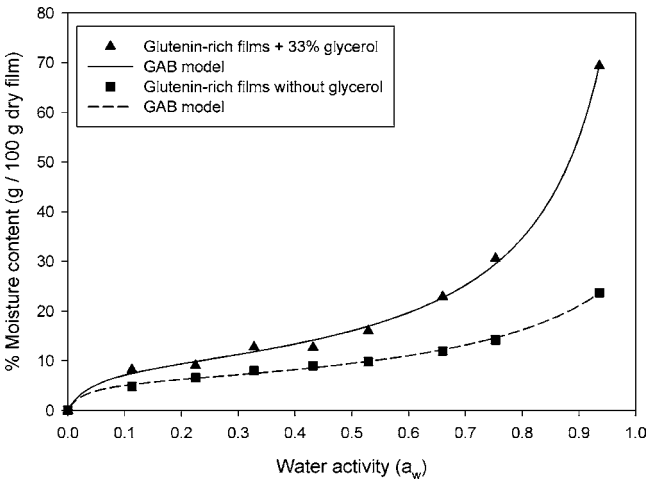

Figure 6. Experimental moisture sorption isotherms and curves predicted by the GAB model for glutenin-rich films plasticized with 0 and 33% glycerol at 23 °C.

The GAB model (see eq 2 under Materials and Methods) is one of the most widely accepted models used in the field of food science (*37*, *38*) and in product development such as the preparation of protein- and polysaccharide-based films (*39*−*42*). This model was thus chosen for the mathematical description of the sorption isotherms over the range of $a_w = 0.11$−0.94. It is generally assumed that a good fit to the isotherm is obtained when E (see eq 3) is <10% (*42*−*45*). The GAB model achieved a good fit to the experimental data; the reported E

Table 3. Water Isotherm Parameters Obtained from the GAB Model

film type	W_m[a]	C[a]	k[a]	E[a] (%)
glutenin	5.8 ± 0.2	42.9 ± 19.7	0.80 ± 0.01	2.44
glutenin-glycerol	9.0 ± 0.4	24.5 ± 13.2	0.93 ± 0.01	3.13
gliadin	6.3 ± 0.4	110.4 ± 10.1	0.80 ± 0.02	2.06
gliadin-glycerol	9.2 ± 0.4	38.8 ± 11.3	0.93 ± 0.01	2.33

[a] See text for definitions.

values are shown in **Table 3** and varied between 2.06 and 3.13. The GAB constants W_m, C, and k for plasticized and unplasticized gliadin- and glutenin-rich films are also given in **Table 3**.

Lewicki (*38*) studied the applicability of the GAB model to biomaterial water sorption isotherms and concluded that the GAB equation was a good description of the sigmoidal shape of isotherms for k and C values falling within the following intervals: $0.24 < k \leq 1$, $5.67 \leq C \leq \infty$. Having the constants in these ranges ensures that the calculated monolayer values differ by not more than ±15.5% from the true monolayer capacity.

In the current study, k values ranged between 0.80 and 0.93 and C values from 24.5 to 110.4. As is shown in **Table 3**, the BET monolayer (W_m) had similar values for unplasticized gliadin- and glutenin-rich films. For films plasticized with glycerol, the W_m value was higher, indicating that the number of active sites that can interact with water molecules increased as a consequence of the presence of the hydroxyl groups of glycerol. The C parameter decreased for films with glycerol, which was previously reported by Cho and Rhee (*46*) for soy protein films and by Kim and Ustunol (*40*) for whey-protein-based films without any physical explanation.

The clustering function values were also calculated using eq 4. Clusters of water molecules are expected to form within the gliadin matrix when a_w is >0.64. For glutenin-rich films, clustering was predicted at $a_w > 0.57$. The higher cohesiveness among glutenin chains compared to gliadins could make the currently active sorption sites less accessible for water binding molecules, thus favoring the self-association of water molecules at lower water activities. Water clustering in glycerol-plasticized films occurred at water activities of 0.51 and 0.48 for gliadin- and glutenin-rich films, respectively, both of which are lower than the predicted values for unplasticized films. Glycerol occupies active sites in the polypeptide network, reducing the availability of such sites for water molecules; thus, it could be expected that the plasticizer promotes water clustering at lower water activities. Similar findings have been reported for collagen films plasticized with polyols (*47*).

Mechanical Properties. The TS and PE of gliadin- and glutenin-rich films as a function of glycerol content are shown in **Figures 7** and **8**, respectively. TS of gliadin-rich films decreased from 8.2 to 0.56 MPa, and PE increased from 6 to 396% for films at glycerol concentrations between 11 and 33% (grams per 100 g of dry protein). Glutenin-rich films presented TS values from 26 to 1.4 MPa and elongation values from 5 to 297% at glycerol concentrations between 11 and 66%. The glutenin-rich fraction yielded films of greater tensile strength and lower elongation values than the gliadin-rich fraction at the glycerol concentrations studied because the presence of intermolecular covalent bonds in glutenins results in a denser and more cohesive polymer network than those formed from gliadins. These findings are in agreement with those of Cornec et al. (*47*), who described gliadins as being responsible for the extensibility of gluten and glutenins as the contributors of the elastic properties of gluten.

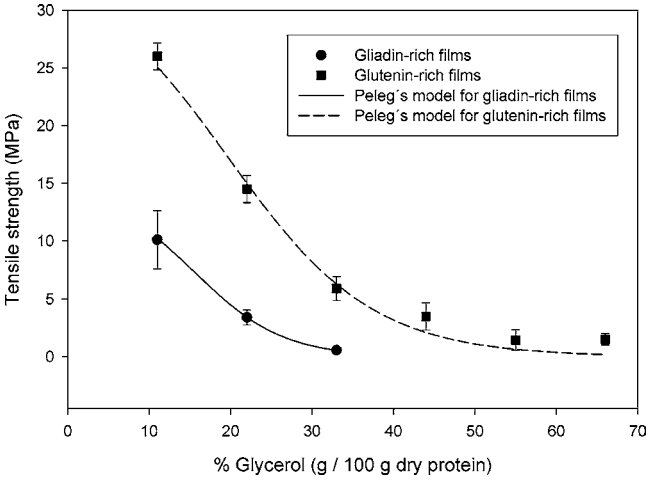

Figure 7. Effect of glycerol on tensile strength of gliadin- and glutenin-rich films.

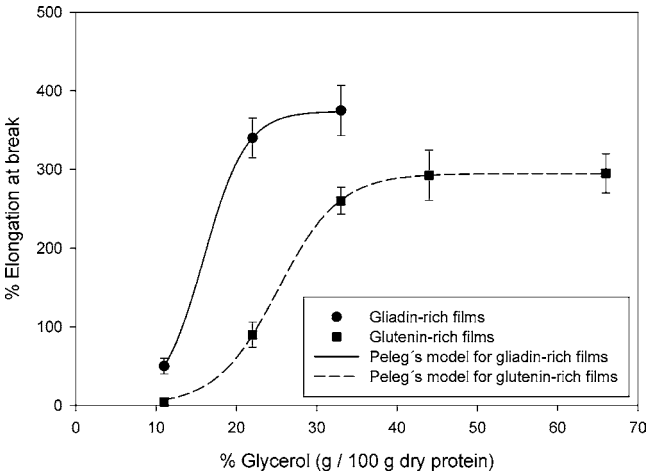

Figure 8. Effect of glycerol on elongation at break of gliadin- and glutenin-rich films.

Effect of Glycerol. Glycerol decreased the tensile strength and increased the elongation of films. This has been reported previously for films made from other proteins such as β-lactoglobulin (*49*) and sodium caseinate (*50*) and from polysaccharides (*51*, *52*). It is well-known that plasticizers decrease the cohesiveness of polymer chains, increasing their segmental mobility and the free volume of the network. As a consequence, films become weaker and more extensible. Tensile strength and elongation of glutenin-rich films were not modified by glycerol addition at concentrations >33% (grams per 100 g of dry protein), which could be attributable to an excess of glycerol molecules that did not interact with the protein matrix. This behavior could not be observed for gliadin-rich films because films lost their integrity at glycerol concentrations >33%.

As can be seen in **Figures 7** and **8**, changes in TS and PE as a function of glycerol were sigmoidal in shape, and Peleg's model (*53*) was used to fit the experimental data. This model describes the mechanical behavior of biomaterials with temperature, water activity, or moisture content at and around the glass transition, and, as in this work, it can also be employed to quantify the effect of plasticizers at a constant temperature (*53*):

$$Y = Y_s/\{1 + \exp[-(X - X_c)/a]\} \quad (7)$$

In eq 7, Y is the calculated value of the mechanical parameter

Table 4. Peleg's Parameters for Tensile Strength (TS) and Elongation at Break (PE) Curves of Gliadin- and Glutenin-Rich Films

parameter	Y_S[a]	X_c[a]	a[a]	R^2[a]
		Gliadin-Rich Films		
TS (MPa)	14.4 ± 3.8	15.8 ± 3.8	−5.4 ± 1.4	0.999
PE (%)	374 ± 13.8	15.9 ± 1.9	2.6 ± 1.0	0.997
		Glutenin-Rich Films		
TS (MPa)	34.3 ± 2.6	20.1 ± 2.1	−8.8 ± 1.6	0.994
PE (%)	307 ± 8.0	21.3 ± 0.6	3.1 ± 1.0	0.944

[a] See text for definitions.

(tensile strength or elongation) at plasticizer concentration X, Y_s is the value of the mechanical parameter for nonplasticized film, X_c indicates the value for plasticizer concentration that decreases Y to $Y_s/2$, and a is an empirical constant and characterizes the broadness of the variation. The curve-fit parameters are given in **Table 4**, and fitted curves for TS and PE have been represented in **Figures 7** and **8**, respectively. Peleg's model fitted well with the experimental data obtained for the variation in the tensile strength and elongation with glycerol content. TS values at zero glycerol content were 14.4 and 34.3 MPa for gliadin- and glutenin-rich films, respectively, in accordance with the more rigid structure of glutenins. It can be observed from the X_c values given in **Table 4** that the loss of mechanical properties of films occurs at greater glycerol content for glutenin films. It can also be observed in **Table 4** that glutenin films presented TS and PE curves with larger a values that those for gliadin films corresponding with a rather flat shape for the depicted curves.

Conclusions. Gliadin- and glutenin-enriched fractions were obtained from crude wheat gluten, and films were formed by the casting technique. The physical properties of films made from gliadin and glutenin fractions were significantly different.The differences appeared to be related to the high molecular weight of glutenins and the presence of intermolecular covalent bonds among the polypeptide chains. Films made from glutenins were stronger and less extensible, with lower water vapor permeability compared to gliadin-rich films. Gliadin-rich films had a better optical appearance and disintegrated after immersion in water. Moisture-holding capacities of gliadin- and glutenin-rich films were similar, although water clustering occurred at lower water activities for glutenin-rich films. Regarding the lower mechanical resistance and higher transparency of gliadin films, these films could be of interest as edible coatings applied to foods, whereas the stronger, water-resistant glutenin films could be candidates for nonedible free-standing films for packaging of low and intermediate water activity foods.

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