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Functional Properties of Bioplastics Made from Wheat Gliadins Modified with Cinnamaldehyde

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ABSTRACT: Cinnamaldehyde is a naturally occurring α,β-unsaturated aldehyde. Its potential as a natural cross-linker to improve the physical performance of cast wheat gliadin films was evaluated. The cross-linking reaction was found to be dependent on the pH of the reaction medium, with pH 2 as the optimum. The water resistance (weight loss after immersion), mechanical properties (Young's modulus, tensile strength and elongation at break), thermal properties (T_g and decomposition behavior), optical properties and morphology of films were evaluated. Cross-linked films showed high transparency, maintained their integrity after immersion, and displayed significant improvements in tensile strength and Young's modulus without impairment of their elongation properties. These effects, which were proportional to the amount of cinnamaldehyde added, highlight the possible formation of intermolecular covalent bonds between "monomeric" gliadins, leading to a polymerized network. Thus, this treatment could provide a new alternative to the toxic cross-linkers commonly employed and so extend the use of gliadin films.

KEYWORDS: bioplastics, gliadin films, cinnamaldehyde, protein cross-linking

INTRODUCTION

The use of renewable raw materials for the manufacture of biodegradable and environmentally friendly plastics is a new technological goal for efficient and sustainable development. Biobased polymers made from different proteins have been researched and developed over the past 20 years for a number of purposes, including the formation of films and coatings for packaging applications. Attention has focused on plant proteins such as wheat gluten, corn zein and soy, and animal proteins such as casein, whey, collagen, keratin and egg albumen. These biomaterials are not intended to replace traditional plastics but to target niche markets in which their short lifecycle is suitable. In food packaging, applications in fresh or minimally processed fruits and vegetables, dairy products, organically grown products, and catering facilities are good candidates.[1] These biopolymers have also led to great interest in the development of sustained release devices for active packaging purposes.

Protein-based films possess moderate mechanical properties in comparison to conventional synthetic films, and good oxygen barrier properties at low or intermediate relative humidities exceeding those of polyolefins.[2] Nevertheless, one of their greatest limitations as packaging materials is their loss of mechanical and barrier properties at high relative humidity levels. In order to overcome this water sensitivity, some modification of the protein is required.

Since amino acids have a large number of reactive side groups (NH_2, COOH, SH, etc.) that are susceptible to physical, chemical or enzymatic modification, covalent cross-linking of polypeptide chains provides a means for improving the physical integrity of these networks and consequently enhancing the functionality of the resulting films.

The food value of wheat is well recognized, as shown by the large volume of wheat-derived breakfast cereals and different types of breads and pasta. However, wheat is now also well established as a raw material for the manufacture of starch. The economics of starch manufacture from wheat depend on the returns from the gluten, the proteinaceous material comprising gliadins and glutenins obtained as a byproduct of isolating the starch from the wheat flour. Wheat gluten has a market in bakery products, especially for protein enrichment, and in the breakfast-cereal industry. As the markets for wheat starch increase, manufacturers are looking to expand the outlets for the concomitant gluten production. This situation, and the general interest in developing environmentally friendly products based on renewable resources, has prompted investigation on nonfood uses for wheat gluten. Wheat gluten presents important viscoelasticity, thermoplasticity, film-forming and other properties which endow this biomaterial with a noteworthy potential for a wide range of technological applications, particularly as a food packaging material.[3] However, gluten presents poor solubility in water and in ethanolic solutions; in addition high molecular weight glutenin subunits tend to form large aggregates hindering gluten processability. In this sense gliadins, with lower molecular weight, have been selected due to their ready extractability in ethanolic solutions in which they show long-term stability and excellent film-forming properties.

Previous studies on gluten proteins have shown that gliadins and glutenins can be exploited separately in order to maximize their range of industrial uses.[4] Films made from gliadins are very glossy and transparent but have poor mechanical resistance and lose their integrity upon immersion in water, which limits their use in specific industrial applications such as packaging materials.[4] Gliadin films obtained by casting have been subjected to thermal curing treatments[5] and have been blended with other renewable polymers that possess superior mechanical strength, such as chitosan,[6] with the aim of improving their functional properties. Chemical agents such as cysteine[7−9] and cross-linking

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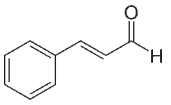

Figure 1. Chemical structure of cinnamaldehyde.

agents such as formaldehyde, glutaraldehyde and glyoxal,[10,11] 1-ethyl -3-(3-dimethylaminopropyl)carbodiimide/*N*-hydroxysuccinimide[9] and epichlorhydrin[12] have also been employed. However, the inherent toxicity of some of these compounds has to be considered when these materials are used in food packaging applications.

Cinnamaldehyde is a naturally occurring aromatic α,β-unsaturated aldehyde derived from cinnamon that has antimicrobial properties.[13] Due to its chemical structure (Figure 1), it could also act as a cross-linking agent for proteins. However, it is uncertain which proteins or other macromolecules react with cinnamaldehyde, and among those that do, which groups have the greatest potential to react.[14]

The aim of this study is to explore the potential of cinnamaldehyde as a cross-linking agent to improve the physical performance of gliadin films.

MATERIALS AND METHODS

Materials. Crude wheat gluten (≥80% protein, 7% fat and 8% moisture content on a dry basis), glycerol, ethanol, hydrochloric acid and cinnamaldehyde (3-phenylprop-2-enal), all laboratory grade, were supplied by Sigma (Madrid, Spain).

Gliadin-Rich Fraction Extraction from Wheat Gluten. The gliadin-rich fraction was extracted from wheat gluten according to the method described by Hernandez-Munoz.[15] Briefly, 100 g of wheat gluten was dispersed in 400 mL of 70% (v/v) ethanol/water mixture, stirred overnight at room temperature and centrifuged at 5000 rpm for 20 min at 20 °C. The supernatant containing the gliadin-rich fraction was collected and used as the film-forming solution.

Chemical Modification of Gliadins. The capacity of cinnamaldehyde to modify gliadins and improve the physical performance of the resulting films was studied in relation to the pH of the gliadin solution. For this purpose 5% (g/100 g of dry protein) of cinnamaldehyde was incorporated into the gliadin solution prior to adjustment of its pH to 1, 1.5, 2, 2.5, 3, 4, or 5 or maintenance of the original pH 6 of the gliadin solution. Glycerol was added as a plasticizer at 25% (g/100 g of dry protein), and the solution was stirred for 1 h at room temperature. The films were cast, dried at 37 °C for 24 h and conditioned at 23 °C and 50% relative humidity (RH). The effectiveness of the cinnamaldehyde at the different pH levels was evaluated by measuring the films' resistance to disintegration in water. In order to establish the pH value at which the loss of proteinaceous film material in water is minimal, the protein solubility of the films which were resistant to water was measured as indicated below. The physical properties of the cinnamaldehyde-modified gliadin films were then determined at the pH at which the films maintained their integrity in water and the protein solubility was minimal; the effect of the cinnamaldehyde content on the properties of the films was evaluated at cinnamaldehyde concentrations of 1.5, 3 and 5% (g/100 g of dry protein).

The samples were coded as follows: G (gliadin), *X*C, where *X* is the percentage of cinnamaldehyde (C), and _pH*Y*, where *Y* is the pH of the reaction.

Film Thickness. The thickness of the films was measured using a micrometer (Mitutoyo, Kanagawa, Japan) with a sensitivity of ±2 μm. The mean thickness was calculated from measurements taken at ten different locations on each film sample.

Protein Solubility. Protein solubility of the films in phosphate buffer was determined by the bicinchoninic acid protein assay kit (Sigma, Madrid, Spain).[16] The film samples were stored in a desiccator over silica gel for 10 days before being immersed in test tubes containing 10 mL of 0.1 M sodium phosphate buffer (pH 7). The tubes were shaken at 180 rpm at 25 °C for 24 h. Aliquots of 1 mL from each test tube were centrifuged at 13000*g* for 10 min, and 0.1 mL of each supernatant was added to 2 mL of the protein determination reagent and heated at 37 °C for 30 min. After cooling to ambient temperature, the absorbance of the solutions was measured at 562 nm in comparison with a reagent blank. Protein content was calculated from a standard curve constructed using bovine serum albumin solution standards (from 0 to 1000 μg/mL). Protein solubility was expressed as milligrams of protein dissolved in the buffer per gram of dry film. Three replications were carried out for each film and the experiment was repeated three times with reproducible results.

Film Color. Film color was measured using a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan) set to D65 illuminant/10° observer. Film specimens were measured against the surface of a standard white plate, and the CIELAB color space was used to obtain the color coordinates L^* [black (0) to white (100)], a^* [green (−) to red (+)] and b^* [blue (−) to yellow (+)]. The color was expressed using the polar coordinates $L^*C^*h^\circ$, where L^* is the same as above, C^* is the chroma and h° is the hue angle. Simple transformations were used to convert $L^*a^*b^*$ coordinates to $L^*C^*h^\circ$ coordinates:

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

$$h^\circ = \arctan\left(\frac{b^*}{a^*}\right) \quad (2)$$

Eight measurements were taken of each sample, and three samples of each film were measured.

Mechanical Properties. A universal machine Mecmesin model MultiTest 1-*i* (Landes Poli Ibérica, S.L., Barcelona, Spain) equipped with a 100 N static load cell was used to evaluate the maximum tensile strength (σ_m), percentage of elongation at break (ε_b) and Young's modulus (E) of the films according to ASTM standard method D882.[17] The films were conditioned at 50% and 75% RH for at least 1 week prior to testing. Film samples were cut into strips 2.54 cm wide and 10 cm long. The grip separation was set at 5.08 cm and the cross-head speed at 25 mm·min^{-1}. At least 10 replicates from each sample were tested. The tensile properties were calculated from the plot of stress (tensile force/initial cross-section area) versus strain (elongation as a fraction of the original length).

Weight Loss. Film specimens (3 × 3 cm^2) were dried in a desiccator over silica gel for ten days, at which point a moisture content close to zero was assumed. The dry films were weighed accurately (initial dry weight, W_i) and immersed in test tubes containing 10 mL of 0.1 M sodium phosphate buffer (pH 7) with or without 2% 2-mercaptoethanol (2-ME). The tubes were shaken at 180 rpm at 25 °C for 24 h. The films were then removed from the buffer solutions, and the remaining water was eliminated from the surface with absorbent paper. The films were weighed and placed in the desiccator again until they reached a constant weight (final dry weight, W_f). The percentage weight loss (WL) of the films was calculated from the following equation:

$$\text{WL } \% = \frac{W_i - W_f}{W_i} \times 100 \quad (3)$$

Modulated Differential Scanning Calorimetry (MDSC). Measurements of the glass transition temperature (T_g) of gliadin films made without glycerol and conditioned at 0% RH at 23 °C were determined by modulated differential scanning calorimetry. It is well-known

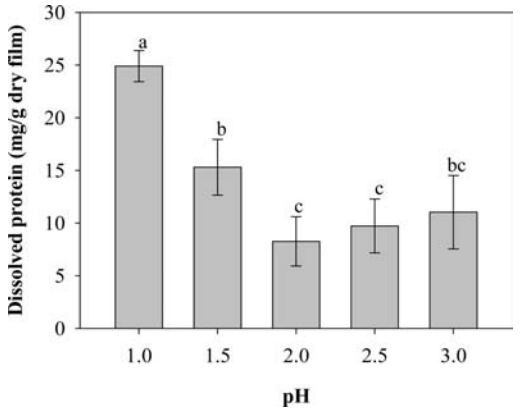

Figure 2. Effect of the pH of the film-forming solution on the protein solubility of water-resistant films made with 5% of cinnamaldehyde after 24 h of immersion in phosphate buffer at pH 7 and 25 °C. Different letters indicate significant differences between means ($p \leq 0.05$) according to the Tukey test.

that, when using conventional DSC, during the first heating of gluten proteins an endothermic peak is superimposed on the glass transition phenomenon, preventing correct measurement of the glass transition temperature. This peak can be eliminated during the second heating, but the protein can be modified as a result of thermal cross-linking. A more reliable measurement of T_g can be obtained using MDSC. This technique makes it possible to separate reversing thermal events from nonreversing ones in a single scan and thus to separate T_g from the endothermic relaxation in gluten proteins. The glass transition of the films was measured using a TA Instruments DSC Q2000 (TA Instruments Inc., New Castle, DE, USA) equipped with Universal Analysis 2000 software. Nitrogen was used as a purge gas at a flow rate of 50 mL/min. Temperature calibration of the instrument was performed with indium. The heating rate was 2 °C/min, the modulation period was 60 s and the amplitude of modulation was 0.32 °C. The glass transition temperature was recorded from the inflection point of the reversing heat flow signal. The films were dried over P_2O_5 at 23 °C for 2 weeks before testing. Dry samples of approximately 5 mg were placed in aluminum pans with inverted lids to achieve optimum thermal conductivity. These were sealed, punctured three times and kept over P_2O_5 for a further week prior to scanning. All samples were measured in triplicate.

Thermogravimetric Analysis (TGA). TGA of gliadin films without glycerol was carried out using a Mettler Toledo TGA/SDTA/851 (Mettler Toledo, Columbus, OH, USA). Samples of approximately 10 mg were heated from room temperature to 800 at 10 °C/min and held at an isotherm for 3 min. The TGA data were plotted as the weight percentage versus temperature, and the decomposition temperature was obtained from the first derivative of weight percentage versus temperature (DTGA).

Morphology. The films were fractured under liquid nitrogen, and their cross-section surface morphology was studied by scanning electron microscopy (SEM) using a Hitachi model S-4100 with a BSE Autrata detector and an EMIP 3.0 image capture system (Hitachi, Madrid, Spain). A copper cube was used as a support for the films, which were fixed with double-sided carbon tape and silver paint. The samples were coated with gold−palladium under a vacuum in a sputter coating unit.

Statistical Analysis. Statistical analysis of the results was performed with SPSS commercial software (SPSS Inc., Chicago, IL, USA). A one-way analysis of variance (ANOVA) was carried out. Differences between means were assessed on the basis of confidence intervals using the Tukey test at a level of significance of $p \leq 0.05$. The data were graphically plotted with Sigmaplot software (Systat Software Inc., Richmond, CA, USA).

RESULTS AND DISCUSSION

pH Effect on the Cross-Linking Capacity of Cinnamaldehyde. The water resistance in phosphate buffer at pH 7 was determined for films modified with 5% cinnamaldehyde and cast from solutions with a pH of between 1 and 6. The films produced at pHs of between 1 and 3 maintained their integrity after immersion in water whereas those prepared at pHs of between 4 and 6 lost their physical integrity and became insoluble masses aggregated in a transparent buffer. Figure 2 shows the film protein solubility of the films that were resistant to water. It can be seen that the pH 1 and 1.5 films presented the greatest protein solubility, which could be due to a possible protein degradation occurring as a result of acid hydrolysis of peptide bonds. In contrast, the lowest protein solubility value was obtained for films made at pH 2; however, there were no significant differences ($p > 0.05$) in the protein solubility of films made at pHs of between 2 and 3. This study shows that there is an optimal pH at which cinnamaldehyde acts as a cross-linker for gliadins and that this could be considered to be between pH 2 and pH 3.

The effect of the reaction medium pH on the color of the films measured by the $L^*C^*h^\circ$ parameters is shown in Figure 3, where it can be observed that the color depends on the pH of the film-forming solution. Untreated gliadin films cast at pH 6 presented a pale yellow color whereas cinnamaldehyde-treated films cast in the pH 2 to 6 range presented a yellow-green color, with hue angles falling in the second quadrant $(-a, +b)$, at around 105°. Gliadin films prepared at pH 6 without cinnamaldehyde presented the lowest chroma. The addition of cinnamaldehyde to gliadins cast at pHs of between 2 and 3 gave rise to films with similar chromaticity, and at higher pHs the intensity of the films' color increased gradually with the pH, which could indicate the appearance of conjugated double bonds which account for color. The low pH films (1, 1.5) developed chroma values similar to those made at pH 5 and pH 4 respectively but presented lower hue values than the films with higher pH values. In general, all the films were very transparent as shown by their high L^* values (around 87). The only significant difference ($p \leq 0.05$) was found in those films modified with cinnamaldehyde at pH 6, which exhibited slightly lower luminosity.

Following these results, the film-forming solution was adjusted to pH 2 before studying the cross-linking effect of cinnamaldehyde on the functional properties of gliadin films. The effect of cinnamaldehyde was studied at concentrations of 1.5, 3 and 5% (G1.5C_pH2, G3C_pH2, G5C_pH2). Two control films were prepared: gliadin films at pH 6 (G_pH6) and gliadin films at pH 2 (G_pH2).

Film Color. The L^*, C^* and h° parameters of gliadin films modified with cinnamaldehyde at pH 2 and of the control films cast at pH 2 and 6 are shown in Table 1. The films made from untreated gliadins at pH 2 and 6 were very transparent, as indicated by their high L^* values, and presented a slight yellowish color. The transparency of the films was not significantly altered by treatment with different amounts of cinnamaldehyde. Compared with the controls, the cinnamaldehyde-treated films all developed a similar yellow-green color, and their chromaticity increased with the amount of cinnamaldehyde incorporated.

Mechanical Properties. The stress−strain $(\sigma-\varepsilon)$ curves of the control and cinnamaldehyde-treated gliadin films are depicted in Figures 4A and 4B, which correspond to measurements taken at 50% and 75% RH respectively. As can be observed in

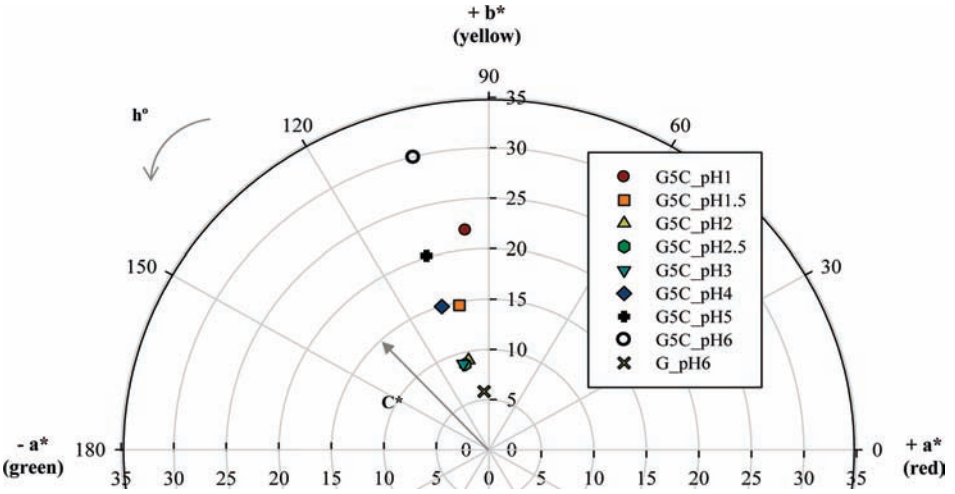

Figure 3. Effect of the pH of the film-forming solution on the $L^*C^*h^\circ$ coordinates of films made with 5% cinnamaldehyde and a control film (G_pH6).

Table 1. Color Coordinates $L^*C^*h^\circ$ of Gliadin Films[a]

sample	L^*	C^*	h°
G_pH6	87.6 ± 0.3 a	5.82 ± 0.58 a	94.4 ± 0.4 a
G_pH2	88.8 ± 0.9 a	5.23 ± 0.76 a	94.4 ± 1.0 a
G1.5C_pH2	88.0 ± 0.5 a	6.24 ± 0.58 a	100.5 ± 0.6 b
G3C_pH2	87.8 ± 0.5 a	7.31 ± 0.61 b	101.5 ± 0.4 c
G5C_pH2	87.9 ± 0.2 a	9.12 ± 0.17 c	102.2 ± 0.2 d

[a] Reported values are means of three different films and eight replicates in each film ± standard deviation. Any two means in the same column followed by a different letter are significantly different ($p \leq 0.05$) according to the Tukey test.

Figure 4A, the control films cast at pH 2 (G_pH2) and pH 6 (G_pH6) presented low σ_m and high ε_b, which is in accordance with the viscous nature of gliadins.[18] The rupture of interpeptidic noncovalent interactions (hydrophobic interactions and hydrogen bonding) due to equibiaxial extension and the formation of new associations allows considerable deformation to occur.[19] Chemical treatment with cinnamaldehyde at pH 2 modified the typically viscous behavior of the gliadin films, giving rise to a different mechanical profile. The stiffness of the films increased, and they presented a greater elastic component. The σ_m of modified gliadin films gradually increased with the amount of cinnamaldehyde incorporated into the film-forming solution, indicating the formation of a cross-linked protein network. Strain hardening was observed after the plateau zone which follows the elastic region. In polymers, strain hardening is due to the straightening of polymer segments between entanglements: the progressive orientation of polymer chains under equibiaxial extension in the direction of flow entails a continued increase of the tensile strength.[19] The new anchor points created in the cross-linked gliadin films produced this phenomenon during the stretching process.

The mechanical properties of non-cross-linked films at 75% RH could not be measured due to the high moisture sensitivity of the gliadin control films. The addition of water generated a high level of plasticity in the polymeric matrix that hindered their manipulation. In Figure 4B the mechanical profiles of cross-linked films conditioned at 75% RH can be observed. The great plasticization of films caused by environmental humidity reduces the intermolecular forces between the polypeptide chains because of the disruption of the hydrogen bonding, which increases the chain segmental mobility and the free volume of the network.[20,21] Consequently, the films become weaker and more extensible.[22]

The mechanical parameters (σ_m, E, and ε_b) of the control and treated films conditioned at 50% and 75% RH are shown in Table 2. The gliadin control films conditioned at 50% RH all presented similar values for these mechanical parameters. The incorporation of cinnamaldehyde into the film-forming solution at pH 2 gave rise to a considerable increase in the σ_m and the E, and a decrease in the ε_b of gliadin films. The σ_m and elasticity of the films increased with the percentage of cinnamaldehyde, suggesting a greater degree of cross-linking in the protein matrix. Thus, both the σ_m and the E of the gliadin films made with 5% cinnamaldehyde and conditioned at 50% RH increased 6-fold compared to the control films while the ε_b only decreased 1.5-fold, and the film continued to exhibit good elongation properties. The σ_m values for the cross-linked gliadin films with 3% (7.7 ± 0.8 MPa) and 5% (9.9 ± 1.2 MPa) cinnamaldehyde were higher than those obtained for cross-linked gliadin films with 40% epichlorohydrin (6.5 ± 0.6 MPa),[12] or 4% formaldehyde, glutaraldehyde or glyoxal (6.5 ± 0.7 MPa, 2.8 ± 0.8 MPa, and 2.1 ± 0.2 MPa respectively).[11] The cross-linked films conditioned at 75% RH followed a similar pattern regarding σ_m and E parameters, but the great plasticization effect caused by water brought about a 2-fold reduction in the σ_m of these films with respect to those conditioned at 50% RH, and the E decreased dramatically, around 16-fold, compared to the cross-linked films conditioned at 50% RH. Although the ε_b of the cross-linked films conditioned at 75% RH could be expected to be higher than that of the films conditioned at 50% RH, this increase was only observed for the films treated with 1.5% cinnamaldehyde whereas the ε_b of the films with higher levels of cinnamaldehyde was similar to the level of those conditioned at 50% RH.

Weight Loss. The control films (G_pH2, G_pH6) could not be recovered after immersion in an aqueous medium because they lost their integrity. The lack of intermolecular covalent bonds between the polypeptide chains in the film matrix was responsible for the films' low water resistance[4] in spite of the low solubility of gliadin proteins in water. Nevertheless, due to the high content of nonpolar amino acids and the low content of

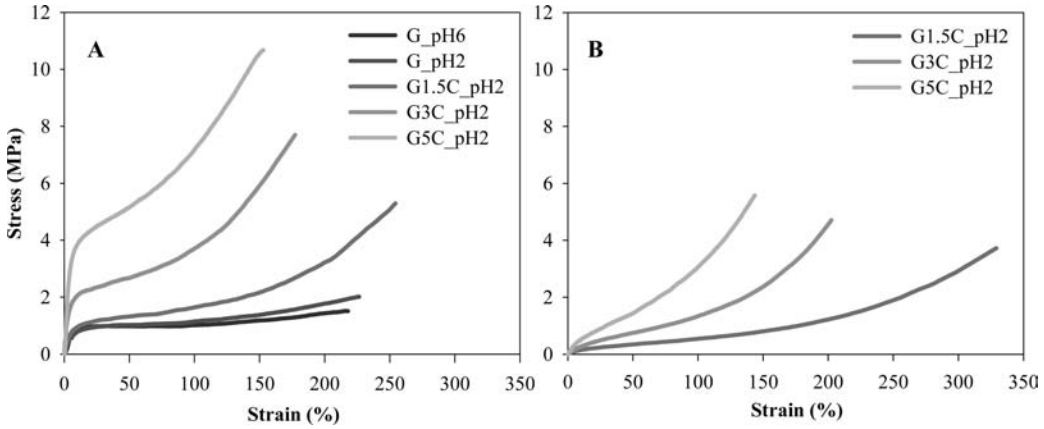

Figure 4. Stress−strain curves of gliadin films at 50% RH (A) and 75% RH (B).

Table 2. Mechanical Properties of Films Tested at 50% and 75% RH[a]

	σ_m (MPa)		ε_b (%)		E (MPa)	
sample	50% HR	75% HR	50% HR	75% HR	50% HR	75% HR
G_pH6	1.60 ± 0.24 a		226 ± 32 cd		24.3 ± 4.9 ab	
G_pH2	1.63 ± 0.39 a		212 ± 30 bcd		19.3 ± 7.7 a	
G1.5C_pH2	5.23 ± 0.83 b	3.55 ± 0.42 a	248 ± 14 d	319 ± 15 c	34.9 ± 6.5 b	2.10 ± 0.74 a
G3C_pH2	7.74 ± 0.83 c	4.63 ± 0.59 b	180 ± 8 ab	197 ± 11 b	63.9 ± 21.7 c	4.67 ± 0.83 b
G5C_pH2	9.93 ± 1.21 d	5.11 ± 0.90 c	146 ± 12 a	144 ± 10 a	112.1 ± 10.9 d	6.73 ± 1.41 c

[a] σ_m: Maximum tensile strength. ε_b: Elongation at break. E: Young's modulus. Reported values are means of 10 replicates ± standard deviation. Any two means in the same column followed by a different letter are significantly different ($p \leq 0.05$) according to the Tukey test.

Table 3. Weight Loss (%) after 24 h Immersion at 25 °C in Phosphate Buffer at pH 7 and Phosphate Buffer Containing 2% 2-ME[a]

	weight loss (%)	
sample	phosphate buffer pH 7	phosphate buffer pH 7 + 2% 2-ME
G1.5C_pH2	21.43 ± 0.68	20.53 ± 1.38
G3C_pH2	21.08 ± 0.43	19.88 ± 1.22
G5C_pH2	20.10 ± 1.63	20.23 ± 0.75

[a] Reported values are means of triplicates of three different films ± standard deviation. No significant differences between means ($p > 0.05$) in the same column were found by the Tukey test.

polar ionizable amino acids present in gliadins,[3] the films did not dissolve. The films made from the gliadin solution with cinnamaldehyde at pH 2 (G1.5C_pH2, G3C_pH2 and G5C_pH2) maintained their integrity after 24 h of immersion in water.

Table 3 shows the weight loss of the different films in a buffer medium at pH 7 and in the same medium with 2% 2-ME. In the buffer, the loss of weight of the gliadin films treated with different amounts of cinnamaldehyde was around 20%, without any significant differences being found between them ($p > 0.05$). Adding 2-ME to the buffer did not change this pattern, which suggests that the possible formation of disulfide bonds during the drying process does not play a major role in the water resistance of films. The loss of weight of the films was mainly due to the diffusion of hydrophilic glycerol into the aqueous medium and, to a lower extent, the loss of polypeptide chains from the reticulated matrix. The loss of glycerol was confirmed not only by the lack of flexibility of the films after drying but also by glycerol accounting for 20% of the dry weight of the film (20 g of glycerol/100 g of dry film).

Modulated Differential Scanning Calorimetry (MDSC). Polymer processing can result in internal molecular stresses which are relieved on reheating. The release of these stresses appears as a small endothermic relaxation peak, which can even occur after the glass transition. The close proximity of the endotherm to the glass transition can prevent the correct evaluation of T_g. In standard DSC, thermal history effects can be eliminated by heating the material once above the T_g, slowly cooling it before evaluation, and then heating it a second time to measure the T_g value. Nevertheless, this type of pretreatment could induce changes such as curing or cross-linking that would alter the results. MDSC, however, separates the thermodynamic and kinetic contributions in a single scan.[23]

Gliadin proteins are amorphous biopolymers capable of undergoing a glass transition from a glassy to a rubbery state. Apart from their 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 the extent of cross-linking. For this test, the films were made without glycerol since the aim of the thermal analysis was to observe whether cross-linking imparts some modification to the T_g of proteins cast into films without any additional effects of glycerol or water. As can be seen in Table 4, the T_g of the pH 6 gliadin control film (G_pH6) had a value of 164 ± 3 °C, in

Table 4. Thermal Properties[a]

sample	T_g (°C)	dec T (°C)
G_pH6	164.0 ± 3.0 ab	323.0 ± 0.5 c
G_pH2	158.4 ± 2.4 a	302.1 ± 2.0 a
G1.5C_pH2	164.2 ± 0.2 ab	306.7 ± 0.5 ab
G3C_pH2	168.7 ± 1.5 bc	309.4 ± 5.8 ab
G5C_pH2	172.9 ± 0.1 c	313.7 ± 3.1 b
cinnamaldehyde		211.6 ± 0.2

[a] Glass transition temperature (T_g) obtained by MDSC and peak decomposition temperature obtained by TGA. Reported values are means of three replicates ± standard deviation. Any two means in the same column followed by a different letter are significantly different ($p \leq 0.05$) according to the Tukey test.

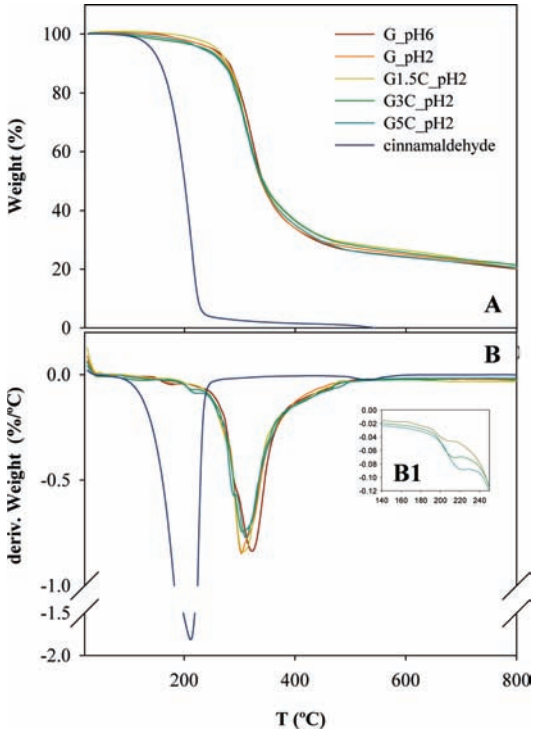

Figure 5. (A) Thermogravimetric (TGA) and (B) derivative thermogravimetric (DTGA) profiles of gliadin films and cinnamaldehyde. (B1) DTGA detail of the region of cinnamaldehyde degradation, cross-linked films only.

agreement with those obtained by several authors.[24,25] For the film cast at a lower pH (G_pH2), the T_g decreased significantly ($p \leq 0.05$). This change in the T_g of the protein could be related to denaturation. At pHs far from the isoelectric point, for example 8.1 for gliadins,[26] side-chain repulsion of induced net charges leads to conformational changes in the structure due to possible unfolding, rupture of hydrogen bonds and alteration of hydrophobic interactions,[27,28] facilitating chain mobility and prompting a lower T_g. Modification of gliadins with cinnamaldehyde at pH 2 produced the expected increase in T_g as the concentration of cinnamaldehyde increased, which can be explained by a restriction of polymer chain mobility due to increases in both the cross-linking density and the molecular weight of the polymer.

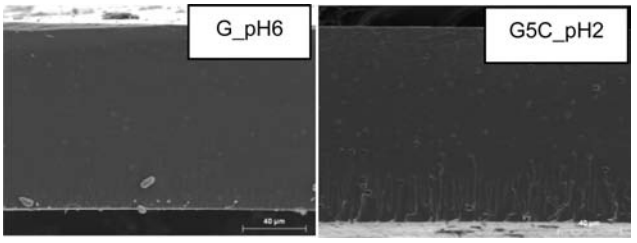

Figure 6. SEM micrograph of the cryofractured cross-section of films G_pH6 and G5C_pH2 (magnification ×600).

Thermogravimetric Analysis (TGA). All the samples showed a one-step decomposition profile with a single transition temperature, as demonstrated by TGA (Figure 5A) and the derivative plots (DTGA) (Figure 5B). The degradation onset temperatures were very similar in all the films, starting at approximately 245 °C, and resulted in a char residue of approximately 20%. Table 4 shows the peak decomposition temperatures of the gliadin films and of cinnamaldehyde. The G_pH6 control film showed a peak decomposition temperature of 323 ± 0.5 °C, slightly lower than that found by Song et al.[12] for films with 20% glycerol (331 °C). The peak decomposition temperature (302 ± 2 °C) of the control film cast at pH 2, G_pH2, was significantly lower ($p \leq 0.05$), showing that it possessed lower thermal stability than the films made at pH 6. This may be attributed to protein denaturation at low pH. Compared with G_pH2, the incorporation of cinnamaldehyde at pH 2 led to increases in the degradation temperature of these films in proportion to the amount of cross-linker added. The peak decomposition temperature of cinnamaldehyde is 212 ± 0.2 °C, with a sharp drop in its decomposition profile. Figure 5B1 shows a detail of the DTGA of the cross-linked films. A slight slope can be detected in the region of cinnamaldehyde decomposition as a function of cinnamaldehyde content. This small loss of weight could be attributed to the free cinnamaldehyde which does not participate in the formation of the cross-linked matrix.

Morphology. Figure 6 shows SEM images of cryofractured cross sections of the G_pH6 control film and the film treated with 5% cinnamaldehyde at a low pH (G5C_pH2). This type of fracturing process allows soft materials to be cut without surface deformation, providing useful information on the structure of the polymer, the formation of different phases and the distribution of the added component. The control films presented a smooth surface with a nonporous structure, and no phase separation was appreciated. No differences were observed compared to the films formed when cinnamaldehyde was added to the film-forming solution at pH 2, indicating great miscibility of cinnamaldehyde with gliadins.

The present work has proved the suitability of cinnamaldehyde as a natural cross-linker for gliadin films, presumably due to the formation of intermolecular covalent bonds among polypeptide chains that polymerize the "monomeric" gliadins and reticulate the protein matrix. The cross-linking capacity of cinnamaldehyde has been found to be dependent on the pH of the reaction medium, with pH 2 being the optimum. The cross-linking effect, which was proportional in its intensity to the amount of cinnamaldehyde added, was evidenced by a great improvement in the mechanical strength of the films without impairment of their elongation properties; complete resistance after water immersion with negligible loss of weight attributable

to protein; and an increase in the T_g compared to the control films. The use of a natural compound such as cinnamaldehyde is a valuable alternative to toxic cross-linking agents for improving the physical performance of renewable films made from gliadins and so extending their use in food packaging applications, as well as in other areas of interest such as agrochemistry, pharmacy, cosmetics, etc.

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ABBREVIATIONS USED

2-ME, 2-mercaptoethanol; MDSC, modulated differential scanning calorimetry; MW, molecular weight; E, Young's modulus; ε_b, elongation at break; RH, relative humidity; SEM, scanning electron microscope; T_g, glass transition temperature; TGA, thermogravimetric analysis; σ_m, maximum tensile strength; WL, weight loss

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