Properties of Chemically and Physically Treated Wheat Gluten Films

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Chemical (vapors of formaldehyde), physical (temperature, UV and γ radiation), and aging treatments were applied to wheat gluten films. Changes in film mechanical properties, water vapor permeability, solubility, and color coordinates were investigated. An aging of 360 h led to a 75 and 314% increase in tensile strength and Young's modulus, respectively, and a 36% decrease in elongation. Severe thermal (above 110 °C, 15 min) and formaldehyde treatments highly improved the mechanical resistance of the films. Under these conditions, up to 376 and 654% increase in tensile strength and Young's modulus and up to 66% decrease in elongation have been observed. Water solubility was only slightly modified, whereas water vapor permeability was not affected. Color coordinates of films heated above 95 °C changed to a great extent. An almost total insolubilization of proteins in sodium dodecyl sulfate occurred for heat- and formaldehyde-treated films, due to the modification of protein network leading to changes in properties of the films.

Keywords: *Wheat gluten; edible film; biodegradable film; chemical cross-links; heat curing; radiation; aging*

INTRODUCTION

The use of proteins for the fabrication of bioplastics has been recently reviewed by Cuq et al. (1998). Numerous plant proteins, such as wheat gluten, corn zein, soy, peanut, and cottonseed, and animal proteins, such as milk casein, whey, albumin, collagen, gelatin, keratin, and myofibrillar proteins, are commonly proposed as raw materials to form either edible or biodegradable packaging films (Cuq et al., 1998; Gennadios et al., 1994; Guilbert, 1986). They form amorphous three-dimensional structures stabilized mainly by noncovalent interactions. A wet process commonly referred as "casting" and a dry process, using the thermoplastic properties of proteins under low water conditions, are used to prepare protein films (Cuq et al., 1998). Many studies refer to the film-forming properties of wheat gluten proteins (Ali et al., 1997; Anker et al., 1972; Aydt et al., 1991; Gennadios and Weller, 1990; Gennadios et al., 1993a,b; Gontard et al., 1992, 1993; Herald et al., 1995). These films are water-resistant and possess selective oxygen and carbon dioxide barriers properties that could be useful in the preservation of fresh and minimally processed vegetables (Gontard et al., 1996). However, because of their hydrophilic nature, proteins films have poor water vapor barrier properties, which limit their use. Furthermore, their mechanical properties and the water barrier properties are strongly affected by the presence of water or other plasticizers (Gontard et al., 1993).

To widen the end-uses of these films, their functional properties must be improved. One of the solutions is to choose appropriate casting conditions. Gontard et al.

(1992) and Herald et al. (1995) showed that gluten films made at low pH from an ethanol solution were stronger than films obtained from alkaline conditions. Under those high pH conditions, the reduction of the disulfide bonds to sulfhydryl groups is favored, thus allowing the film to stretch further. Recently, Morel et al. (2000) showed that formation of disulfide bonds by thiol oxidation occurred during wheat gluten film storage, even in conditions (temperature and relative humidity) under which the mobility of molecules is reduced. Therefore, mechanical properties of the films might be changed with film aging, depending on the rate of thiol oxidation during film drying and storage.

Trying to cross-link the proteins is a second method to obtain stronger and less permeable films. The improvement of film functional properties by cross-linking induced by chemical (Galietta et al., 1998; Gueguen et al., 1998; Marquié et al., 1995; Parris and Coffin, 1997), thermal (Alcantara et al., 1998; Ali et al., 1997; Gennadios et al., 1996; Herald et al., 1995; Miller et al., 1997; Stuchell and Krochta, 1994), and radiation (Brault et al., 1997; Gennadios et al., 1998) treatments was also investigated by several authors. Most of them were applied as "pretreatment", meaning that changes occurred in the film-forming solution. Some attempts to modify the film properties were also performed using "posttreatments" (applied on the film). Most of these treatments were applied on films made from different protein sources. Hence, a comparative evaluation of the different treatments was very difficult.

The objective of this study was to compare the effects of aging and some various chemical or physical treatments (applied as "posttreatments") on the properties of wheat gluten films. The properties studied were stress and elongation at break, Young's modulus, water vapor permeability, solubility in water, color, and total sodium dodecyl sulfate (SDS) extractable proteins.

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MATERIALS AND METHODS

Film Preparation. Wheat gluten (N × 5.6 = 76.5%) (Amylum Group, Aalst, Belgique) concentration (12.8 g (d.b.)/100 mL solution), ethanol concentration (32 mL/100 mL solution), and pH of the solution (4, adjusted with acetic acid) were chosen according to Gontard et al. (1992). They observed that, by casting, such a formulation produced a resistant, homogeneous, and transparent film without insoluble particles or excessive increase of water vapor permeability. In our experiment, the gluten was first mixed with a reducing agent (sodium sulfite, 30 mg) and water (∼20 g) for 15 min before adding ethanol. The plasticizer (glycerol, 3 g/100 mL of solution) was added, as described by Gontard et al. (1992) to the ethanolic medium. The film-forming solution was then heated at 50 °C under magnetic stirring and immediately poured and spread onto a crystal PVC plate (30 × 40 × 0.8 cm) placed on a level surface with the help of a thin-layer chromatography spreader bar (0.8 mm) (Braive instruments, Checy, France). The film-forming solution was dried for 20 h at 25 °C in a ventilated oven (Memmert ULE 600, German) before peeling off from the PVC support.

Film Treatments. For the studies of the effect of aging, freshly cast films were conditioned for 48, 144, and 360 h at 20 °C and 60% RH prior to property evaluations. For all other treatments, the time between the end of casting and property measurement was fixed to 144 h at 60% RH and 20 °C, to test films with the same aging conditions. Posttreatments were always conducted on a film aged for 48 h and then stored again at the same temperature and RH to reach a total aging of 144 h. For γ radiation posttreatment, aging time before film characterization was 360 h due to an extra delay to treat the material in the Commissariat à L'Energie Atomique laboratory (Cadarache, France). Properties of posttreated wheat gluten films were always compared with nontreated films ("control") with the same aging as the posttreated films (144 or 360 h). Properties of control films of the same aging time, but from individually prepared and cast film-forming solutions, were reproducible (ratio standard deviation to mean value <20%).

Formaldehyde was chosen as a model chemical cross-linker. Because of the difficulty in removing formaldehyde excess from films soaked in an ethanolic solution of formaldehyde (Gueguen et al., 1998), posttreatment by vapors was chosen. In addition, soaking may remove plasticizer, causing the films to become brittle. Thus, the film was placed for 24 h in a hermetic box containing one liter of an ethanolic solution of formaldehyde (10% v/v). UV treatments were applied to films at 0.25 and 1 J/cm² doses using a UV oven (254 nm, Amersham Life Science, U.K.).

Heat treatment was performed with a molder (Techmo, France) comprising two heating plates adjusted according to the exact film thickness but without any applied pressure. Films, placed on a Teflon sheet, were heated at 80, 95, 110, and 125 °C for 15 min and at 140 °C for 1.5 and 15 min. The boiling point of pure glycerol at atmospheric pressure is 290 °C but drops substantially in the presence of water (Gregory, 1991). To evaluate the potential loss of glycerol during thermal treatments, glycerol was quantified in the film before and after heating by high-pressure liquid chromatography (HPLC). A water extract, obtained by immersion of the film in water (24 h with continuous stirring followed by a 24-h period in a cold room without stirring), was dosed on a RSil-R5C18-25F HPLC column (250 × 4.6 mm, Interchim, France) and eluted by water (1 mL/min). Detection was carried out by refractometry.

Treatment by γ radiation was performed by the Commissariat à L'Energie Atomique (Cadarache, France) using ⁶⁰Co source on films previously cast in our laboratory. Three irradiation doses were tested: 10, 20, and 40 kGy.

Film Properties. *Mechanical properties* of films were investigated on dumbbell-shaped specimens of 75 mm overall length [5A type, standard ISO 527-2: 1993 (F)] with a texturometer TAXT2 (Rheometer, Champlan, France). Elongation at break tests were performed for a 20 mm/min elongation speed. Measurements were done in an environmental chamber at 20 °C and 60% RH, on film previously stored for 48 h in the same conditions. Film thickness was measured at five points with a hand-held micrometer (Braive Instruments, Checy, France). Tensile strength was calculated by dividing force at break by the initial cross-sectional area of the film, and elongation at break was expressed in percentage of the initial length of the elongating part of the specimen (gauge length, $L_0 = 20$ mm). Given tensile strength and elongation at break values ($\Delta L/L_0 \times 100$) are the mean of 10 replicates. Young's modulus was determined as the slope of the linear regression performed on the first points (∼10) of the stress–strain curves.

Film color was determined using a Minolta Chroma Meter (CR 300, Minolta Chroma Co., Osaka, Japan). A CIE Lab color scale was used to measure the degree of lightness (L^*), redness ($+a^*$) or greenness ($-a^*$), and yellowness ($+b^*$) or blueness ($-b^*$) of the films. The instrument was standardized using a Minolta calibration plate with D_{65} illuminant ($L^* = 97.71$; $a^* = -0.1$; $b^* = +1.89$). Values given in this report are means of five measurements on three areas for each film.

Film solubility in water was defined by Gontard et al. (1992) as the percentage of dry weight solubilized after 24 h immersion in water. The test was performed as described by these authors. The percentage of initial dry matter of each film was determined by drying for 24 h in an oven at 104 °C. Two disks of film (30 mm diameter) were cut, weighed, and immersed in 50 mL of water containing sodium azide (0.02% w/v) to prevent microbial growth. After the specimens were immersed for 24 h at room temperature with agitation, they were removed and dried for 24 h in an oven at 104 °C, to determine the loss of dry matter.

Water vapor permeability (WVP) tests were conducted using the modified ASTM (1989) procedure used by Gontard et al. (1992). The film was sealed in a glass permeation cell containing water (100% RH). The cells were stored at room temperature in ventilated desiccators containing silica gel. After steady-state conditions were reached, the cells were weighed at regular time intervals, and WVP of the film was calculated as

$$\text{WVP} = \frac{wx}{At(p_2 - p_1)} \text{ (mol·m·m}^{-2}\text{·s}^{-1}\text{·Pa}^{-1})$$

where w is the weight loss of the cup (g), t is the time of gain (s), x is the film thickness (m), A is the area of exposed film (m²), and $p_2 - p_1$ is the vapor pressure differential across the film (Pa). Three measurements were carried out for each film.

Total Extracted Proteins (TEP). Film proteins were extracted with a sodium phosphate buffer, which contained SDS (1%), and sonicated as described by Redl et al. (1999). The extraction was performed for 80 min instead of 24 h. Extracted proteins were fractionated by size-exclusion HPLC, and TEP was estimated from chromatographic profiles as described by Redl et al. (1999).

Statistical Analysis. The results were compared by variance analysis. A Duncan's multiple range test ($p < 0.05$) was used to determine significance between treatments. Statgraphics Plus software was used for this purpose.

RESULTS AND DISCUSSION

Influence of Posttreatments on Mechanical Properties. Typical tensile stress–strain curves obtained with some posttreatments (formaldehyde, heat-curing, UV 0.25 J/cm²) are presented in Figure 1. All curves displayed two parts, characterized by a high difference in slope. The value of the stress at the slope's change can be defined as the yield stress. We noticed that 140 °C and formaldehyde treatments gave close values of tensile strength and elongation at break. However, the two stress–strain curves differed especially regarding the yield stress (5.7 and 3.8 MPa for thermal and formaldehyde treatments, respectively). This is evidence of a difference in the cross-linking mechanisms occur-
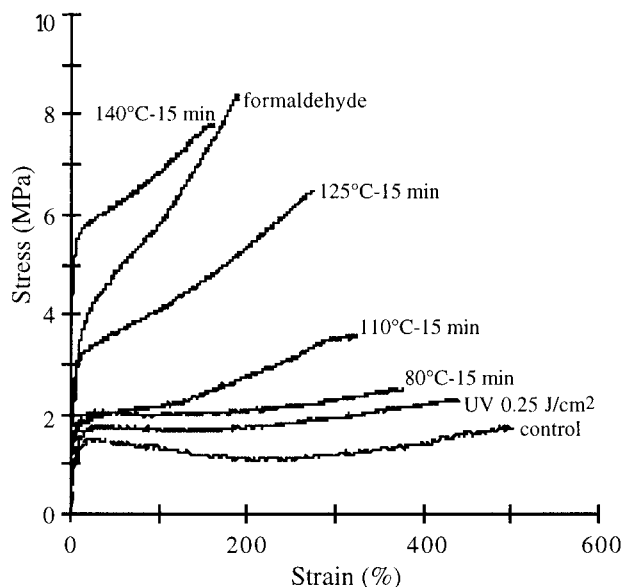
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Figure 1. Stress−strain curves, until break, of posttreated wheat gluten films.

Table 1. Mechanical Properties of Wheat Gluten Film as a Function of Aging[a]

aging (h)	mechanical properties		
	tensile strength (MPa)	elongation (%)	Young's modulus (MPa)
48	1.2 ± 0.2 a	604 ± 102 a	7 ± 3 a
144	1.7 ± 0.3 b	501 ± 46 b	13 ± 3 b
360	2.1 ± 0.5 c	384 ± 82 c	29 ± 4 c

[a] Reported values are means of 10 replicates ± standard deviation. Any two means in the same column followed by same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test.

ring during the two treatments. It is interesting to note that for temperature treatments, curves differed in the second slope between the control and the 80 and 110 °C treated films, with stress yield remaining almost the same. These changes in the second slope were similar to those described by Yeoh and Fleming (1997) for rubber with increasing curative levels, showing that the temperature increase probably modified the cross-links density between proteins in the wheat gluten film. Between the 110, 125, and 140 °C treatments, the second slope remained almost constant, while yield stress was multiplied three times. Important differences in tensile strength and elongation at break (Figure 1) were noticed among treatments, which are discussed below (Tables 1−3).

Aging. When film aging increased from 48 to 360 h, a 75% increase in tensile strength, a 314% increase of stiffness (Young's modulus), and a 36% decrease in elongation are observed (Table 1). Previous work (Morel et al., 2000) on wheat gluten film showed that storage duration and conditions (temperature and RH) of the films greatly influenced thiol oxidation. The increase in thiol oxidation during aging, leading to the formation of protein polymers of large molecular size, might be responsible for the changes in mechanical properties of films. Thus, it appears in this study that aging time has to be taken into consideration prior to characterization of film properties.

Formaldehyde Treatments. Because of posttreatment with formaldehyde, the elongation of wheat gluten films

Table 2. Mechanical Properties of Posttreated Gluten Films[a]

treatments	mechanical properties		
	tensile strength (MPa)	elongation (%)	Young's modulus (MPa)
control	1.7 ± 0.3 a	501 ± 46 a	13 ± 3 a
formaldehyde	8.1 ± 0.8 g	190 ± 27 f	70 ± 26 d
temp (°C)			
80 (15 min)	2.4 ± 0.4 b	391 ± 58 cd	29 ± 13 bc
95 (15 min)	2.5 ± 0.3 bc	386 ± 75 cd	30 ± 11 bc
110 (15 min)	3.1 ± 0.4 c	327 ± 58 de	37 ± 10 c
125 (15 min)	6.3 ± 0.5 e	275 ± 12 e	40 ± 14 c
140 (15 min)	7.3 ± 1.2 f	170 ± 26 f	98 ± 16 e
140 (1.5 min)	4.2 ± 1.1 d	326 ± 49 de	37 ± 8 c
UV radiation (J/cm²)			
0.25	2.0 ± 0.3 ab	424 ± 97 bc	18 ± 6 ab
1	2.0 ± 0.3 ab	478 ± 70 ab	15 ± 4 a

[a] Reported values are means of 10 replicates ± standard deviation. Any two means in the same column followed by same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test.

decreased (62%), the stiffness (Young's modulus) increased (438%), and the tensile strength increased (376%) (Figure 1, Table 2). Pea (Gueguen et al.,1998), cotton seed flour (Marquié et al., 1995), and zein (Parris and Coffin, 1997) films pre- and/or posttreated by formaldehyde also showed an increase in tensile strength, from 165 to 470%. Upon formaldehyde treatment, a 30−60% decrease in elongation was commonly detected in zein and pea films, whereas an increase was observed for cottonseed flour (Marquié et al., 1995). Pea films posttreated by formaldehyde (Gueguen et al., 1998) gave changes in mechanical properties (470% increase in tensile strength and 60% decrease in elongation) comparable to those observed on gluten films. The effect of formaldehyde treatment could be different depending on the polymer treated. In contrast to the soaking method employed by Gueguen et al. (1998), vapor exposition procedures (this study) gave very reproducible results.

To evaluate the solubility of proteins after formaldehyde treatment, untreated (control) and formaldehyde posttreated films were sonicated in SDS solution. Sonication allows the solubilization of all native wheat gluten proteins, by breaking down the size of the larger insoluble aggregates (Singh et al., 1990). When compared to the untreated film, which was almost totally soluble in SDS after sonication (soluble proteins: 93.7 ± 4% of total protein), the formaldehyde posttreated films contained less than 1% of SDS-soluble proteins. This confirms that changes in the mechanical properties of formaldehyde-treated films is due to the reinforcement of the network by covalent linkages (e.g., methylene bridge). Formaldehyde cross-linking of film proteins probably occurs through free amino groups (lysine), as already pointed out by Marquié et al. (1997).

Temperature Treatments. When the temperature increased from 80 to 140 °C, an increase in tensile strength (41 to 329%) and a decrease in elongation (22 to 66%) were observed (Figure 1, Table 2). When the temperature increased from 110 to 125 °C, there was a 2-fold increase in tensile strength, whereas elongation decreased progressively when films were heated from 80 to 140 °C.

The inverse relationship observed between stress and strain at break (Figure 1) is classically attributed to differences in plasticizer content (Sánchez et al., 1998).

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In this study, the glycerol loss of heat-cured films never exceeded 11% of its initial content. When referring to the results reported by Gontard et al. (1993) on similar wheat gluten films, such a difference in glycerol concentration would have little effect on changes in film mechanical properties. This confirms the predominant effect of thermal treatment.

Heat treatment resulted in aggregation of proteins by cross-linking (hydrophobic and disulfide bonding) (Ali et al., 1997; Gennadios et al., 1996; McHugh et al., 1994; Weegels et al., 1994). The aggregation of proteins of heat-cured films has been assessed by SDS solubility measurements. Heating a wheat gluten film at 140 °C for 15 min resulted in almost total insolubility of the proteins (soluble proteins: $2.27 \pm 0.04\%$ of total proteins). When gluten was heated at 80 °C for 30 min, Weegels et al. (1994) observed an 11−32% decrease in protein extractability in SDS. This was attributed to an aggregation of the glutenins.

Decreasing the time of treatment from 15 to 1.5 min at high temperature (140 °C) still resulted in a large increase in the tensile strength and a decrease in the elongation of wheat gluten film as compared to the values of control film. The 140 °C-1.5 min treatment gave values comparable to those obtained after a 110 °C−15 min treatment. As already pointed out by Gennadios et al. (1996), who investigated soy protein isolate films heated at 80 and 95 °C from 2 to 24 h, the interaction between temperature and exposure time is significant. However, wheat gluten films heated for shorter times (15 min, our study) and 2 h (Ali et al., 1997) at 95 °C instead of 80 °C resulted in few or no changes in their mechanical properties.

UV Radiation. Over the range of dose used, UV posttreated films showed no or few changes in their mechanical properties (Figure 1, Table 2). According to Gennadios et al. (1998), the efficiency of UV radiation depends on the protein source and in particular on amino acid compositions and molecular structures. When investigating the effect of UV radiation as posttreatment on soy protein films with a lower dose (0.0104 J/cm^2) than that used in our experiments, these authors observed an increase in tensile strength (65%) and a decrease in elongation (31%). The notable amounts of tyrosine and phenylalanine in soy protein, which may participate in cross-linking under UV radiation, could explain these changes in mechanical properties of the films. In contrast, for UV post-irradiated (0.25 J/cm^2) pea films (Gueguen et al., 1998), no modification of their mechanical properties was detected, as observed in our study with gluten films.

The comparison of the effect of formaldehyde, temperature, and UV radiation on mechanical properties of gluten films clearly showed that the most efficient treatments were the vapors of formaldehyde and the 140 °C−15 min temperature treatment, followed by the 125 °C−15 min and the 140 °C−1.5 min treatments. According to Parris and Coffin (1997) and Marquié et al. (1995), formaldehyde used as pretreatment was the most efficient cross-linker as compared to other chemicals. UV radiation and, to a lesser extent, the less drastic temperature treatment (80 °C-15 min) were least effective in modifying the mechanical properties of the films.

Gamma radiation increased tensile strength and decreased the elongation at break (Table 3). No clear effect of dose irradiation was observed except for

Table 3. Mechanical Properties of γ-Irradiated Gluten Films[a]

	mechanical properties		
	tensile strength (MPa)	elongation (%)	Young's modulus (MPa)
control γ	2.1 ± 0.5 a	384 ± 82 a	29 ± 4 a
10 kGy	3.0 ± 0.7 b	261 ± 82 c	52 ± 15 a
20 kGy	2.6 ± 0.4 b	344 ± 45 ab	36 ± 6 a
40 kGy	2.7 ± 0.4 b	297 ± 54 bc	38 ± 7 a

[a] Reported values are means of 10 replicates $\pm$ standard deviation. Any two means in the same column followed by same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test.

Table 4. Water Vapor Permeability (WVP) and Solubility in Water of Wheat Gluten Film as a Function of Aging[a]

aging (h)	film properties	
	WVP (10^{-12} mol m^{-1} s^{-1} Pa^{-1})	solubility (%)
48	6.6 ± 1 a	25 ± 1 a
144	7.6 ± 1 a	22 ± 1 b
360	6.4 ± 0.1 a	26 ± 1 a

[a] Reported values are means of three (WVP) and two (solubility) replicates $\pm$ standard deviation. Any two means in the same column followed by same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test.

elongation at break. A 10 kGy dose resulted in a 32% decrease in elongation. Increasing the radiation dose above 10 kGy reduced the effect observed. This could be attributed to a decrease in insoluble glutenin polymers as shown by Köksel et al. (1998) on wheat flour. They showed that increasing irradiation from 2.5 to 20 kGy led to a progressive decrease in the amount of largest polymers of glutenin, giving lower average molecular size by depolymerization and/or breakdown of covalent linkages. The positive effect of γ radiation on the mechanical properties observed on gluten films irradiated by 10 kGy could be explained by the formation of dityrosine upon irradiation. Hence, these cross-linkages within polypeptide macromolecules would lead to changes in mechanical properties as suggested by Brault et al. (1997) who studied caseinates films made from γ-irradiated film-forming solution.

Influence of Posttreatments on Water Vapor Permeability and Solubility in Water. The properties of water vapor permeability and solubility in water obtained for each treatment (aging, chemical, and physical treatments) applied on gluten films are presented in Tables 4−6.

All the gluten films maintained their integrity (i.e., did not break apart) even after 24 h of incubation in water with gentle stirring. For the control, the value of $22 \pm 1\%$ corresponded to the loss of plasticizer (23−24% w/w). Values of $\approx 26\%$ have been obtained for the 360 h aging, the 95 °C heated film and γ control and γ-treated films, probably meaning that all the glycerol was lost with an additional part of the film disrupted in water. Temperature-treated films showed solubility around 17−19%. This decrease in solubility can be because (i) their glycerol content, determined by HPLC, was slightly lower (21−22% w/w) than those of control film and (ii) glycerol diffusion might be slower due to the increasing protein cross-linking, as observed by Galietta et al. (1998) on formaldehyde-treated whey-protein-based film. The insolubility of the gluten matrix in water is well-known (Gontard et al., 1992). For comparison, soy or whey protein-based films gave higher

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Table 5. Water Vapor Permeability (WVP) and Solubility in Water of Posttreated Wheat Gluten Films[a]

	film properties	
treatments	WVP[b]	solubility (%)
control	7.6 ± 1 a	22 ±1 ab
formaldehyde	6.7 ± 0.5 a	17 ± 4 c
temp (°C)		
80 (15 min)	6.8 ± 0.4 a	22 ± 1 b
95 (15 min)	6.3 ± 0.7 a	26 ± 1 a
110 (15 min)	6.6 ± 0.8 a	19 ± 1 bc
125 (15 min)	7.6 ± 0.4 a	17 ± 0 c
140 (15 min)	6.4 ± 0.2 a	19 ± 0 bc
140 (1.5 min)	6.7 ± 1.3 a	20 ± 4 bc
UV radiation (J/cm²)		
0.25	7.9 ± 0.5 a	23 ± 2 ab
1	7.5 ± 0.4 a	21 ± 0 bc

[a] Reported values are means of three (WVP) and two (solubility) replicates ± standard deviation. Any two means in the same column followed by same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test. [b] In 10^{-12} mol m^{-1} s^{-1} Pa^{-1}.

Table 6. Water Vapor Permeability (WVP) and Solubility in Water of γ Irradiated Wheat Gluten Films[a]

	film properties	
treatments	WVP (10^{-12} mol m^{-1} s^{-1} Pa^{-1})	solubility (%)
control γ	6.4 ± 0.1 a	26 ± 1 b
10 kGy	7.6 ± 0.2 c	27 ± 1 b
20 kGy	7.6 ± 0.5 bc	25 ± 0 b
40 kGy	6.7 ± 0.4 ab	25 ± 2 b

[a] Reported values are means of three (WVP) and two (solubility) replicates ± standard deviation. Any two means in the same column followed by same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test.

water solubility (30 and 41%, with amounts of plasticizer of 20 and 25%, respectively) (Galietta et al., 1998; Stuchell and Krochta, 1994). As a consequence, further reinforcement of the protein network induced by posttreatment led only to a small change in this water solubility test.

No treatment resulted in significant improvement of water vapor permeability (WVP) (Tables 4−6). Some authors observed a decrease in WVP of heat-cured protein films (Ali et al., 1997; Gennadios et al., 1996; McHugh and Krochta, 1994; Miller et al., 1997; Stuchell and Krochta, 1994), generally attributed to the formation of covalent cross-links and to a decrease in hydrophilicity of the proteins. When the temperature increased from 65 to 95 °C for 2 h, the decrease in WVP of wheat gluten films, observed by Ali et al. (1997) never exceeded 17%. For γ irradiation of gluten films (10 and 20 kGy), an increase in WVP of 19% was observed (Table 6). This might be due to degradation of glutenin, as already pointed out. Despite the severe conditions of posttreatments, these results confirm that the resulting cross-linking was not sufficient to significantly improve the water barrier properties of wheat gluten films.

Influence of Posttreatments on Color Coordinates. Variation of color coordinates (L^*, a^*, b^*) is only presented for heat-curing posttreatment (Table 7). No change in color coordinates was observed for other posttreatments. When the temperature increased from 20 to 140 °C, there was a 20% decrease in whiteness (L^*), and 1137 and 307% increases in redness (a^*) and yellowness (b^*), respectively. The main changes occurred at temperatures above 95 °C when films were

Table 7. Color Coordinates of 15 min Heat-Cured Films as a Function of Temperature[a]

	color coordinates		
temp (°C)	L^*	a^*	b^*
20	94.73 ± 0.3 a	−0.81 ± 0.01 a	11.15 ± 0.53 a
80	93.88 ± 0.9 a	−0.80 ± 0.04 a	13.99 ± 0.55 b
95	94.64 ± 0.4 a	−1.01 ± 0.05 a	12.53 ± 1.03 ab
110	91.32 ± 0.6 b	+0.52 ± 0.34 b	25.05 ± 1.38 c
125	79.86 ± 1.7 c	+8.28 ± 1.21 c	41.70 ± 1.98 d
140	75.42 ± 1.4 d	+10.56 ± 1.15 d	45.40 ± 1.25 e

[a] Reported values are means of five replicates ± standard deviation. Any two means in the same column followed by same letter are not significantly ($p > 0.05$) different by Duncan's multiple range test.

heated for 15 min. When the treatment time increased up to 24 h at 95 °C, the modification of color coordinates of wheat gluten films slightly increased, as observed by Ali et al. (1997). The color coordinates of soy protein films heated for 24 h at 95 °C (Gennadios et al., 1996) were not modified as compared to those of the gluten films (Ali et al., 1997), showing the higher sensitivity of gluten films to temperature treatment. When myofibrillar protein-based film was heated at 104 °C for 24 h (Cuq et al., 1996), an increase in the b^* value was found to be similar to that of gluten films heated for 24 h at 95 °C (Ali et al., 1997). The change in color coordinates accompanying the improvement of the mechanical properties of gluten film treated by temperature (see above) might be prejudicial to the film's future use (especially food use).

CONCLUSIONS

Mechanical properties of wheat gluten films can be greatly modified by treatment with formaldehyde or with short time high-temperature heat-curing (>110 °C). These two posttreatments induced drastic protein insolubilization, probably due to an increase in covalent binding between glutenins, leading to changes in the mechanical properties. However, tensile strength always increased at the expense of the film extensibility. Thermal treatments also induced changes in film color, which have to be considered for food application. The use of formaldehyde, which is one of the most efficient posttreatments, will reduce application to the field of biodegradable packaging. Others film properties such as water vapor permeability and solubility in water were less affected, even for the most severe posttreatments. Evidence of the influence of film aging on their mechanical properties has been demonstrated here.

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