

Mechanical and Water Barrier Properties of Glutenin Films Influenced by Storage Time[‡]

PILAR HERNÁNDEZ-MUÑOZ,^{*,†,§} AMPARO LÓPEZ-RUBIO,[§] VALERIA DEL-VALLE,[§]
 EVA ALMENAR,[§] AND RAFAEL GAVARA[§]

School of Packaging, Michigan State University, East Lansing, Michigan 48824, and Institute of Agrochemistry and Food Technology, CSIC, Apartado Correos 73, 46100 Burjassot (Valencia), Spain

The goal of this work was to study the effect of storage time on the functional properties of glutenin films plasticized with selected hydrophilic low molecular weight compounds: glycerol (GL), triethanolamine (TEA), and sorbitol (S). Glutenins were extracted from wheat gluten, and films were cast from film-forming solutions. The glutenin-based films were homogeneous, flexible, translucent, and easy to handle. Films were stored in an environmental chamber at $50 \pm 5\%$ relative humidity and 23 ± 2 °C. Optical, mechanical, and water vapor permeability properties were monitored at regular intervals for 16 weeks. Films plasticized with GL and TEA had similar mechanical and water vapor barrier properties during the first few days of fabrication. Films plasticized with S were stronger, with better water vapor barrier properties. Mechanical and water vapor permeability properties of films plasticized with GL changed dramatically over time, whereas the properties of films plasticized with TEA and S remained stable during storage. Color properties of films plasticized with GL, TEA, and S did not change within the time period studied.

KEYWORDS: Glutenin films; storage time; plasticizers; mechanical properties; water vapor permeability

INTRODUCTION

There is an extensive body of research regarding the use of vegetal and animal proteins such as wheat gluten, soy, corn zein, whey, casein, albumin, collagen, and gelatin as materials for fabrication of edible films and coatings. These studies include many applications in food packaging as described in several reviews (1–5). These materials are attractive because they are biodegradable and come from renewable sources. In general, protein films present good barriers to oxygen and aromas at low to intermediate relative humidity conditions (6, 7), and they are resistant to fats and oils (8). However, because of their hydrophilic nature, proteins do not provide an efficient barrier to water transport.

The strong intermolecular forces of the protein chains make films rigid and brittle. For this reason, plasticizers are commonly incorporated to increase flexibility and toughness. Plasticizers are low molecular weight substances that act by disrupting intermolecular chain forces and increasing the free volume of the polymer network. The final properties of plasticized films depend on the plasticizer's chemical structure, molecular weight, concentration, and affinity with the polymer.

Due to the low molecular weight of these compounds, plasticizers are potential migrants in packaging applications.

Their gradual release from the polymer matrix promotes film aging. As a consequence, some properties of the film could change, decreasing protection for the packaged food. From this point of view, a careful selection of an appropriate plasticizer in the design of a film or coating formula is crucial in order to provide a prolonged effect in the film.

Glycerol (GL) is commonly used as a plasticizer for protein films because it confers the desired flexibility and extensibility to films. However, due to its relatively small molecular size, glycerol migrates from the polymeric matrix and, consequently, the film tends to revert to its natural stiffness within a few days.

Park et al. (9) found that films made from wheat gluten and corn–zein mixtures plasticized with glycerol and poly(ethylene glycol) deteriorated in their mechanical properties within 20 days of storage due to the loss of plasticizers. Gueguen et al. (10) and Viroben et al. (11) studied the aging process of gluten films plasticized with 1,2- or 1,3-propanediol and reported that tensile strength dramatically increased within 1 month as the plasticizers were released from the films. Micard et al. (12) found that wheat gluten films plasticized with glycerol became stronger and less extensible with aging. Similarly, Butler et al. (13) found water barrier and mechanical properties of chitosan films plasticized with glycerol changed during storage. Anker et al. (14) reported that the mechanical properties of whey protein films plasticized with sorbitol remained stable after 4 months of storage but that films plasticized with glycerol became stiffer during the storage time. Cuq et al. (15) found that mechanical and water vapor permeability properties of myofibrillar protein-based films

* Address correspondence to this author at the Institute of Agrochemistry and Food Technology, CSIC, Aptdo. 73, 46100 Burjassot (Valencia), Spain [telephone (+34)963900022; fax (+34)963636301; e-mail pheman@iata.csic.es].

[‡] This work is dedicated to the memory of Prof. Rubén Hernández.

[†] Michigan State University.

[§] Institute of Agrochemistry and Food Technology, CSIC.

Table 1. Physical Properties of the Plasticizers Studied^a

plasticizer type	molecular mass, Da	atmospheric bp, °C	chemical structure
glycerol	92.09	292	HOCH ₂ CH(OH)CH ₂ OH
triethanolamine	149.19	335	(HOCH ₂ CH ₂) ₃ N
sorbitol	182.17		HOCH ₂ [CH(OH)] ₄ CH ₂ OH

^a From *Handbook of Chemistry and Physics*, 68th ed.; CRC Press: Boca Raton, FL.

plasticized with saccharose did not change during a storage time of 8 weeks; the films, however, became yellow and discolored.

To extend the period in which the films remain flexible, a less volatile plasticizer with larger molecules can be used as plasticizer. Sorbitol (S), for instance, makes more stable films than GL. However, because sorbitol is solid at room temperature, it tends to crystallize at the film's surface, conferring an undesirable whitish and partially opaque aspect to the film (14).

Triethanolamine (TEA) is included in the group of substances known as "indirect" food additives by the U.S. Food and Drug Administration. TEA is commonly used as a component of adhesives, coatings, paper, paperboard, and polymers in contact with food. In addition, it is widely used as an ingredient in emulsifiers, thickeners, wetting agents, detergents, and alkalizing agents in cosmetic products; as a chemical intermediate for anionic and nonionic surfactants and surface active agents in household cleaning agents, textiles, herbicides, pharmaceutical ointments, and other products; as a vulcanization accelerator in the manufacture of rubber; and in many other industrial applications. Due to the hydrophilic nature of TEA, which contains in its structure three hydroxyl functional groups and one amino functional group, it is expected that this compound provides chemical compatibility with protein. TEA has a higher molecular weight and boiling temperature than glycerol, as indicated in **Table 1**. Irissin-Mangata et al. (16) observed that gluten films plasticized with TEA had tensile strength and water vapor permeability similar to those of films plasticized with GL. Although TEA provides a plasticizing effect in gluten films similar to that of GL, no studies have been reported describing the time effect on films containing TEA.

The aim of this work was to evaluate the effect of time on the functional properties of glutenin films plasticized with GL, S, and TEA. Color, mechanical, and water vapor barrier properties of glutenin films were measured during 16 weeks of storage at 23 ± 2 °C and 50 ± 5% relative humidity (RH).

MATERIALS AND METHODS

Reagents. Commercial wheat gluten was supplied by Sigma Chemical Co. (St. Louis, MO). Glycerol (GL), triethanolamine (TEA), sorbitol (S), ethanol, and acetic acid, all of laboratory grade, were obtained from Aldrich Chemical Co. (Milwaukee, WI).

Film Formation. Initially, wheat gluten was dispersed in an aqueous solution of 70% (v/v) ethanol, stirred overnight, and centrifuged at 10000g for 30 min at 23°C. After centrifugation, the supernatant (gliadin fraction) was discarded, and the precipitate, containing mostly glutenins, was treated with a 50% ethanol/water solution and 0.05 N acetic acid. The mixture was stirred and heated at 40°C for 1 h. The insoluble portion containing starch and aggregates was eliminated by centrifugation. Ethanol was added to the supernatant up to 70% ethanol and left for 12 h at 2°C in order to precipitate the glutenins. This step allowed the purification of glutenins from residual gliadins that remained in the solution. The glutenins were separated by centrifugation at 10000g for 30 min, and the supernatant, containing residual gliadins, was discarded. The precipitated glutenins were dispersed in ethanol/water 50% (v/v). The pH was adjusted at 5.0 with acetic acid, the mixture

was centrifuged at 10000g. The supernatant collected was the film-forming solution from which the films were cast. The protein content in the film-forming solution was 7.5% (w/w). The plasticizer (GL, TEA, or S) was added to the protein film-forming solution in a proportion of 33 g/100 g of dry protein. For weight loss in water evaluation of films, the plasticizer was added to the film-forming solution in a concentration ranging from 0 to 44% (g/100 g of dry protein). A measured volume of the film-forming solution was poured on a horizontal flat Teflon tray, and water and ethanol were allowed to evaporate. The films were dried at 23 ± 2 °C and 50 ± 5% RH for 10 h and peeled from the casting surface.

Storage Conditions. Films were preconditioned in an environmental chamber at 23 ± 2 °C and 50 ± 5% RH for at least 48 h prior to testing their moisture content, loss of weight in water, water barrier, mechanical, and color properties. The evolution of these properties was measured during the 16 weeks in storage.

Film Characterization. *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.

Equilibrium Moisture Content at 50% RH. Equilibrium moisture content was determined by drying the samples of 500 mg in a vacuum oven at 60 °C for 24 h (17). The moisture content was calculated on a dry basis and reported as the average of three replicates from different batches.

Loss of Weight in Water (LWW) at 23 °C. Specimens were first dried in a desiccator containing dry calcium sulfate. Dry film samples of ~500 mg were immersed in beakers containing 50 mL of distilled water at 23 °C during 24 h with periodical gentle manual agitation. Films were removed from the water and placed back in the desiccator until constant weight. LWW was reported as a percentage of weight loss in water on a dry film basis and was the average of three samples.

Water Vapor Permeability (WVP). Water vapor transmission [kg/(m²·s)] was measured using a Permatran W3/30 (MoCon Inc., Minneapolis, MN) at 23 °C with a gradient of 50 to 0% RH (dry nitrogen) across the film. At least three samples of each film type were measured. Permeability values are reported as WVP coefficient [(kg·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 of elongation at break (PE) of films according to ASTM standard D-882-91 (18). Sample films were cut into 25.4 mm wide strips. The Instron grip separation was set at 50.8 mm and the cross-head speed at 508 mm/min. At least 10 samples of each type of film were evaluated.

Color. Film color was measured using a CR-300 Minolta Chroma Meter (Minolta Camera Co., Ltd., Osaka, Japan). Film specimens were placed on a white standard plate (*L** = 100, and the CIE *L*a*b** coordinates were measured, using D65 illuminant /10° observer. Five measurements were taken in each sample, and three samples of each film were measured.

Statistical Analysis. Statistical analysis of variance (*p* ≤ 0.05) was performed using the MSTeAT-C statistical program (Michigan State University).

RESULTS AND DISCUSSION

Films were prepared according to the indicated procedure. Films containing 33 g of GL, TEA, or S per 100 g of dry protein were produced. The corresponding molar concentrations were 0.36, 0.18, and 0.22 mol/100 g of dry protein, respectively. Film thickness was ~50 μm. Films were homogeneous, flexible, strong, and translucent, with smooth surfaces.

Loss of Weight in Water. In general, glutenins present extremely low solubility in water compared to other proteins such as whey or soy proteins; this is due to the glutenins' high molecular weight and relatively large amounts of amino acids with nonpolar side chains (19). Glutenin films did not show any sign of physical disintegration after immersion in water for 24 h. Nevertheless, unplasticized glutenin films showed a loss

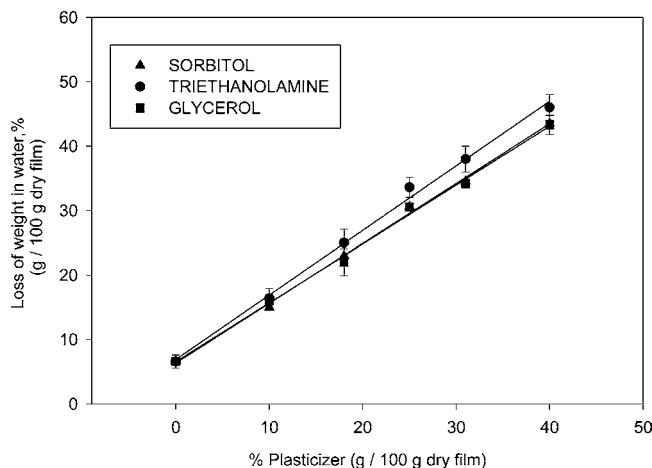


Figure 1. Effect of plasticizer type and content on the loss of weight in water of glutenin films at 23 °C.

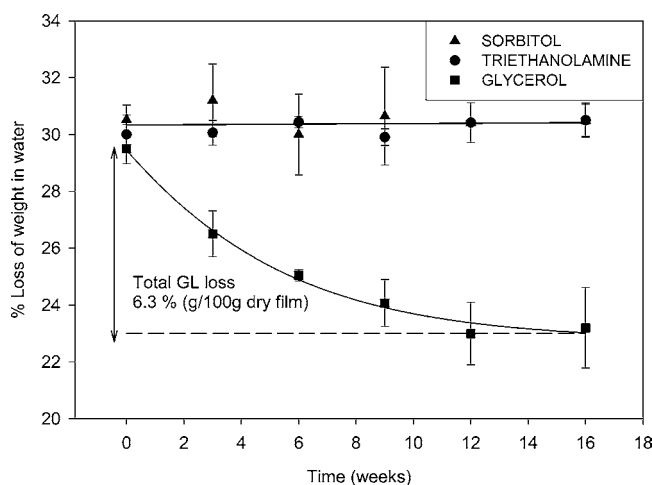


Figure 2. Loss of weight in water of glutenin films initially plasticized with 33% glycerol, triethanolamine, or sorbitol as a function of storage time.

of matter in water at room temperature of $\sim 5\text{--}6$ g/100 g of dry protein. The soluble matter may correspond to nonprotein compounds remaining in the film after casting and low molecular weight polypeptides. In general, the addition of plasticizer increased soluble matter of the films because plasticizers are extracted by water following a first-order linear regression ($R^2 = 0.997, 0.996, \text{ and } 0.998$ for GL, TEA, and S, respectively) as is shown in **Figure 1**. Similar results were reported by Cuq et al. (20) for fish myofibrillar protein-based films plasticized with glycerol, sorbitol, or sucrose. After loss of weight evaluation, film samples were extremely brittle, confirming the diffusion of the plasticizer to the water. Regarding the high insolubility of glutenins in water, the measure of film soluble matter could be considered as an indirect measure of the amount of plasticizer remaining in the film.

Initial soluble matter values of studied plasticized films were ~ 30 g/100 g of dry film in all cases. Nevertheless, the evolution of soluble matter during storage differs among plasticizers. Whereas LWV values remained constant for films containing TEA or S without showing significant differences ($p \geq 0.05$), a significant decrease from 29.5 ± 0.2 to 23.2 ± 1.4 g/100 g of dry film in 16 weeks was observed for films plasticized with glycerol, as **Figure 2** shows. Thus, considering that the loss of weight is mainly attributed to the plasticizer in the film, the amount of GL retained by the polymer after 16 weeks was 23.2 ± 1.4 g/100 g of dry film and, therefore, 6.3 g of glycerol (g/

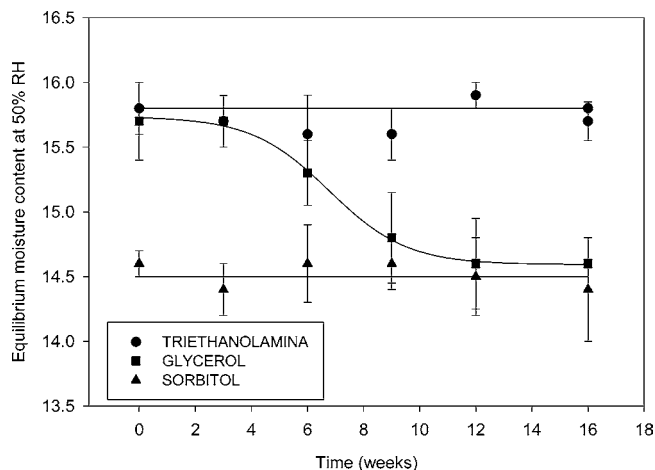


Figure 3. Effect of storage time on equilibrium moisture content at 50% RH of glutenin films initially plasticized with 33% glycerol, triethanolamine, or sorbitol.

100 g of dry film) migrated to the surface of the film and was released during storage. This may indicate that there were two populations of GL molecules in the plasticized films: population I with free molecules and population II with molecules firmly retained in the polymer matrix

Equilibrium Moisture Content at 50% RH. Glutenin films plasticized with GL or TEA had similar initial moisture content of about 15.8 ± 0.2 and $15.7 \pm 0.3\%$ on a dry basis, respectively. The moisture content of films plasticized with S was $14.6 \pm 0.1\%$; this lower value is probably attributable to the lower hygroscopicity of sorbitol (21). As can be seen in **Figure 3**, the moisture content of the films plasticized with GL decreased from 15.7 ± 0.3 to $14.6 \pm 0.2\%$ after 16 weeks of storage. For films containing TEA or S the moisture content remained unchanged without showing significant differences ($p \geq 0.05$). These values are higher than the $9.8 \pm 0.3\%$ for glutenin films without plasticizer. These results confirm that the addition of hydrophilic compounds such as the plasticizers studied in this work to a gluten-based polymeric matrix increases the water-holding capacity of the films and therefore the moisture content. Anker et al. (22) and Mahmoud and Savello (23) reported that initial moisture content of whey protein films plasticized with S and GL increased proportionally to the plasticizer content in the polymer. According to these results, the decrease in moisture content with time in films containing GL may be related to the loss of plasticizer during storage as has been discussed previously.

Water Vapor Permeability. Values of WVP of the films as a function of time are shown in **Figure 4**. Initial WVP coefficients of films plasticized with G or TEA did not differ significantly ($p \geq 0.05$), having values of $(4.3 \pm 0.3) \times 10^{-14}$ and $(4.2 \pm 0.3) \times 10^{-14}$ (kg m)/(m² s Pa), respectively, whereas S-containing film showed a lower WVP value of $(0.67 \pm 0.10) \times 10^{-14}$ (kg m)/(m² s Pa) ($p \leq 0.05$). The lower WVP values of protein films plasticized with sorbitol have also been reported by other authors (24–27). WVP values for films containing TEA or S remained constant for 16 weeks ($p \geq 0.05$), whereas films with GL experienced a significant decrease of 25% in WVP between the 6th and 12th weeks ($p \leq 0.05$) from $(4.3 \pm 0.3) \times 10^{-14}$ to $(3.2 \pm 0.5) \times 10^{-14}$ (kg m)/(m² s Pa). These results might again be related to the loss of glycerol during storage and the associated reduction in water content. In fact, the curve profile of WVP is similar to that of moisture content (see **Figures 3 and 4**).

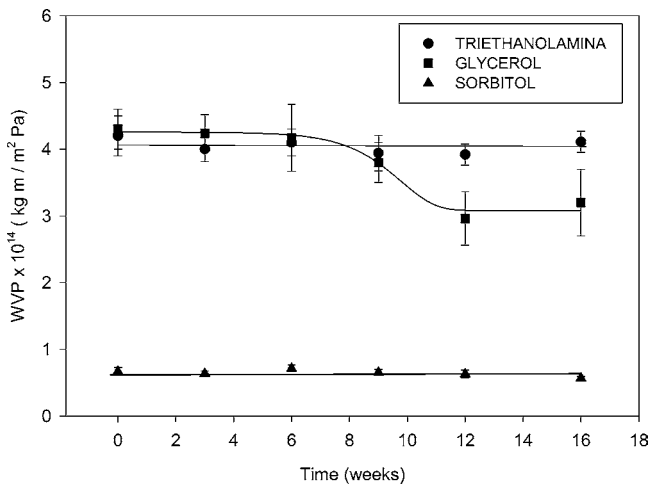


Figure 4. Effect of storage time on water vapor permeability of glutenin films initially plasticized with 33% glycerol, triethanolamine, or sorbitol.

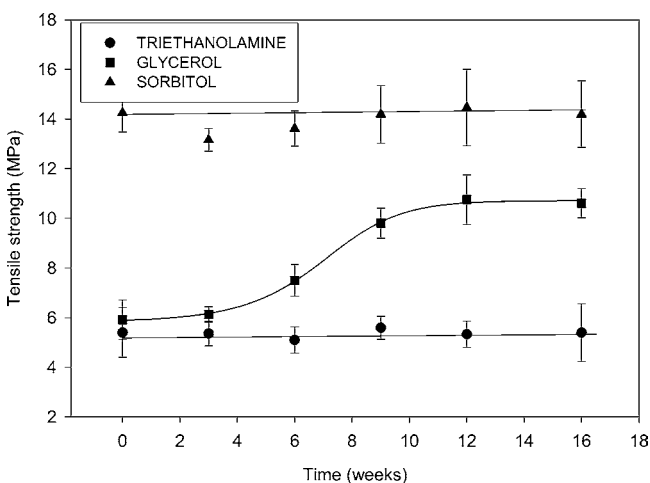


Figure 5. Tensile strength of glutenin films initially plasticized with 33% glycerol, triethanolamine, or sorbitol as a function of storage time.

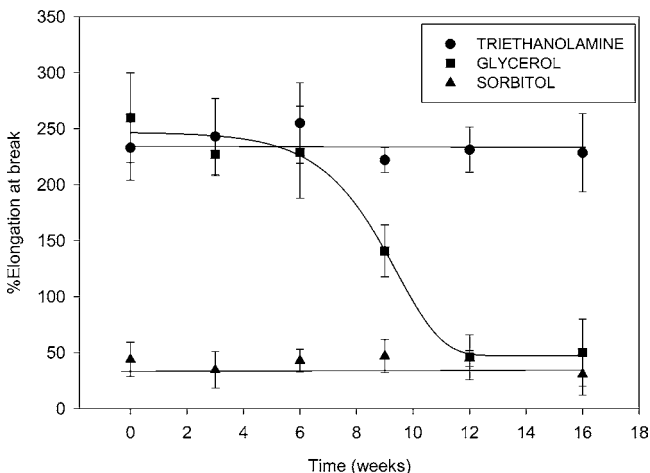


Figure 6. Elongation at break of glutenin films initially plasticized with 33% glycerol, triethanolamine, or sorbitol as a function of storage time.

Mechanical Properties. Tensile strength (TS) and percent of elongation at break (PE) of glutenin films plasticized with GL, TEA, and S are plotted as a function of time in **Figures 5** and **6**, respectively. Films plasticized with GL and TEA had similar initial mechanical values with TS and PE of 5.9 ± 0.8 MPa and $260 \pm 40\%$ and 5.4 ± 1.0 MPa and $233 \pm 29\%$, respectively; no significant differences were observed ($p \geq$

0.05). TS of films plasticized with S was higher than for films containing GL or TEA ($p \leq 0.05$), having a value of 14.2 ± 0.8 MPa; the extensibility of the films plasticized with S was lower, having a PE value of $44 \pm 15\%$ ($p \leq 0.05$). Mechanical properties of films plasticized with TEA or S remained stable after 16 weeks of storage. The PE of films with GL remained constant in the first 6 weeks ($p \geq 0.05$) but dramatically decreased between the 6th and 12th weeks, dropping to $46 \pm 20\%$. The TS of films with GL remained fairly constant in the first 3 weeks ($p \geq 0.05$) to increase to the maximum value of 10.6 ± 0.6 MPa in the following 9 weeks. It appears that after the 12th week, the mechanical properties remained constant. Again, although these results appear to be related to the loss of glycerol, they correlate much better with the evolution of moisture content, as already observed for WVP values.

From these results, TEA appears to be the additive that produces the best plasticization of the glutenin film. The resulting film presents low stiffness and high extensibility. As a consequence of plasticization, the water barrier decreases considerably in films containing TEA. On the other hand, S produces a poor plasticization of glutenin films, with high tensile strength and low elongation at break values and a corresponding good water barrier. Fresh films plasticized with GL present mechanical and water barrier properties similar to those for TEA-containing films, which is attributable to the high hygroscopicity of these compounds. However, properties of films with GL change with time because during storage the loss of GL promotes a decrease in water content and the films become stiffer and more brittle, with an increase in the water barrier, which resembles films containing S.

Another interesting result is that, apparently, the plasticization of the films correlates much better with the water content than with the percentage of plasticizer. The effect on mechanical or barrier properties is clearly dependent on the plasticizer added. However, the moisture content of the sample is also clearly related with the plasticization effect. It seems as if the so-called plasticizers do not produce a direct plasticizing effect but induce this behavior indirectly, as a consequence of increasing the capacity of the film to retain water. That is, water appears to be the actual plasticizer of glutenin films. This is in agreement with the findings of Shaw et al. (21), who reported that the relative effectiveness of glycerol, xylitol, or sorbitol to plasticize whey protein isolate based films was attributable to their hygroscopicities and therefore their capacity to modify the moisture content of the films. Sothornvit and Krochta (28) also reported that the lower water binding capacity of sucrose and sorbitol compared to glycerol was responsible for the lower extensibility of β -lactoglobulin films.

Color Coordinates. Color coordinates L^* , a^* , and b^* of glutenin films plasticized with GL, TEA, or S were evaluated as a function of storage time. All films were translucent with a yellowish color. Films plasticized with GL had L^* , b^* , and a^* average values of 95.5 ± 0.3 , 7.6 ± 0.3 , -0.19 ± 0.02 , respectively. Values for films plasticized with S were 95.3 ± 0.4 , 7.6 ± 0.2 , -0.21 ± 0.03 , which did not show significant color differences with respect to glycerol-containing films. Lower lightness ($L^* = 94.3 \pm 0.2$) and highest greenness ($a^* = -0.38 \pm 0.03$) and yellowness ($b^* = 8.2 \pm 0.3$) values were exhibited by films containing TEA as plasticizer. Storage time did not affect color coordinates of films ($p \geq 0.05$), which were constant for the 16 week period studied.

Conclusions. It can be concluded that GL, TEA, and S were effective plasticizers imparting flexibility to the glutenin films. The initial molar concentrations of GL, TEA, and S content in

the films were 0.36, 0.22, and 0.18 mol/100 g of dry protein, respectively. During the 16 weeks of storage, molar concentrations of TEA and S in the polymer remained constant, while GL was reduced to a final concentration of 0.25 mol/100 g of dry protein. It was apparent that GL had two different molecular populations: one that was lost during the storage time and another that was firmly retained by the polymeric matrix. The loss of two-thirds of the molecules during the first 6 weeks did not greatly affect film properties. As the concentration of GL molecules remaining in the polymer approached 0.25 mol/100 g of dry protein, the moisture content, WVP, and PE at break decreased and the TS increased significantly. Films plasticized with TEA or S maintained these properties throughout the 16 week period. Color coordinates remained stable for all plasticized films during the storage time tested.

Selection of an appropriate plasticizer has to be done in terms of conferring enough flexibility to the film and keeping its functional properties stable for a desirable period of time.

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