Aging of Whey Protein Films and the Effect on Mechanical and Barrier Properties

Martin Anker, Mats Stading,* and Anne-Marie Hermansson

SIK—The Swedish Institute for Food and Biotechnology, P.O. Box 5401, SE-402 29 Göteborg, Sweden

This work focuses on the aging of whey protein isolate (WPI) films plasticized with glycerol (G) and sorbitol (S). The films were cast from heated aqueous solutions at pH 7 and dried at 23 °C and 50% relative humidity (RH) for 16 h. They were stored in a climate room (23 °C, 50% RH) for 120 days, and the film properties were measured at regular intervals. The moisture content (MC) of the WPI/G films decreased from 22% (2 days) to 15% (45 days) and was thereafter constant at 15% (up to 120 days). This affected the mechanical properties and caused an increased stress at break (from 2.7 to 8.3 MPa), a decreased strain at break (from 33 to 4%), and an increased glass transition temperature (T_g) (from −56 to −45 °C). The barrier properties were, however, unaffected, with constant water vapor permeability and a uniform film thickness. The MC of the WPI/S films was constant at ~9%, which gave no change in film properties.

INTRODUCTION

The aging of polymeric materials includes chemical and/or physical changes that occur in the material after exposure to environmental conditions over a period of time. Chemical changes such as oxidation may cause degradation of the polymer chains. Physical changes such as migration of low molecular weight additives affect the film properties and are important to consider when in the design of an appropriate film formula. This migration is a natural process that occurs when low molecular weight substances, such as plasticizers, are added to polymeric films and is difficult to avoid. Generally, migration leads to stiffer and less extendable polymers, which may decrease the protective function of the packaging and consequently reduce the shelf life of the packaged food (*1–3*). The effect on the barrier properties is also important. An increased permeation of oxygen increases vitamin degradation, lipid oxidation, and other oxidative reactions, whereas an increased diffusion of moisture increases the water activity (a_w) in the food. Critical levels of a_w must be maintained in many foods to prevent microbial growth and texture degradation and to minimize deteriorative chemical and enzymatic reactions (*4*). The migration phenomenon may be desirable, however, if the film is intended for use as a carrier of, for example, antioxidants and antimicrobial agents that will migrate into the packed food and prolong its shelf life.

The extensive use of plasticizers in flexible films has spurred studies that have shown that some plasticizers have a strong tendency to migrate into foods under certain conditions (*5–7*). As a result of the high fat solubility of some plasticizers, such as di-(2-ethylhexyl) adipate (DEHA), migration is often more pronounced in foods that have a high fat/moisture ratio, especially where there is a large contact area between the food and the plastic (*5*). Startin et al. (*7*) showed that the highest levels of migration of the plasticizer occurred when there is direct contact with fatty foods such as cheese and cooked meat and that migration is enhanced at higher temperatures such as during cooking. Other factors that influence the migration are the concentration of the additive in the polymer and the hydrophilic and hydrophobic character of the additive. Adcock et al. (*8*) showed that the amount of additive migration is directly proportional to the concentration of additive in the polymer, provided that the additive is readily soluble in the extractant. The authors also found that the presence of a second additive seemed to influence the migration of the first additive and that additives of the same class migrated together in ratios similar to the ratios at which they were found in the extractant. This is important for the present study, in which the plasticizers glycerol (G) and sorbitol (S) were added as separate second additives apart from the first additive, water, basically used as solvent.

Both G and S have been used in our previous investigations (*9–11*) and are among the most common plasticizers added to biopolymeric films. Both plasticizers migrate, and the present paper was thus initiated to identify the effect of this migration on the mechanical and barrier properties of whey protein isolate (WPI) films. The main difference between the two plasticizers with regard to the effect of migration is that S crystallizes on the surface of the WPI films, which gives the films a slightly undesirable appearance, whereas the migration of G is not that easily detected. This is because S is crystalline at room temperature, whereas G is a clear liquid. The time scale for the first signs of crystallization of S on the film surface can be rather long (up to 4 months) and depends on the amounts of plasticizer used. The more plasticizer that is used, the faster the detection. However, if the films are to be used on a short time scale, or used inside foods, the consumer

* Author to whom correspondence should be addressed (telephone +46 31 335 56 00; fax +46 31 83 37 82; e-mail mats.stading@sik.se).

Table 1. Physical Properties of Glycerol and Sorbitol

	glycerol	sorbitol
formula	$C_3H_8O_3$	$C_6H_{14}O_6$
molecular mass (Da)	92	182
form	liquid	powder
melting point (°C)	18[a,c]	98−100[b,c]
boiling point (°C)	290[a,c]	295[d,c]

[a] From Nordling Österman (*43*). [b] From Sigma-Aldrich (*44*). [c] Pressure 100 kPa. [d] From Weast et al. (*45*). [e] Pressure 0.5 kPa.

will not notice this crystallization. Park et al. (*2*) studied wheat gluten and corn zein films and showed that, when G migrated during the storage time of 20 days, the tensile strength increased and the elongation decreased. The authors implied that even when G is well dispersed in the protein matrix, the plasticizer migrates from the bulk to the surface because of the binding limitations between the protein molecules and G. The migration rate was also suggested to be dependent on the types of functional groups, polarity, and structure of the film matrix. This was in agreement with the findings of Park et al. (*12*), who observed the loss of flexibility of corn zein films as an indication of G migration. The authors further stated that the greasy appearance on the surface within a few hours was an indication of the plasticizer migration. Similar results have been reported for the aging of starch-based materials (*13*−*16*). Van Soest et al. (*16*) examined thermoplastic potato starch plasticized with G and water and after 2 weeks of aging. A rapid increase in B-type crystallinity and a change in the mechanical properties were observed. Specifically, the tensile stress of the films increased (from 3 to 7 MPa) while the elongation decreased (from 105 to 55%). Similar results were presented for extruded starch sheets aged for 60 days (*15*), thermoplastic potato starch aged for 90 days (*14*), and barley starch aged for 5 weeks (*13*).

The aim of this paper was to determine the effects of aging on the mechanical and barrier properties of WPI films plasticized with G and S. The films were stored in a climate room at 23 °C and 50% realtive humidity (RH) for 120 days, and the film properties were measured at regular intervals. The effect of aging on the glass transition temperature (T_g) was also studied.

MATERIALS AND METHODS

Materials. WPI was obtained from MD Food Ingredients (Videbaek, Denmark). WPI (Lacprodan PSDI-9224) is a functional WPI used for protein fortification of clinical nutrition products and sports foods. The WPI powder had a dry content of 93 ± 2% protein (N × 6.38), 0.2% fat, 0.2% lactose, 5.5% moisture, and 4.0% minerals and a pH between 6.5 and 7.0 (0.10% solution). The concentration of proteins in the WPI powder was 74% β-lactoglobulin, 18% α-lactalbumin, 6% bovine serum albumin, and 2% immunoglobulins. G and S (>98% purity) were used as plasticizers and were obtained from BDH Laboratory Supplies (Poole, U.K.) and Sigma Chemical Co. (St. Louis, MO), respectively. G and S are colorless, odorless, and sweet-tasting alcohols. Their physical properties are listed in Table 1. G is obtained from oils and fats as a byproduct in the manufacture of soaps and fatty acids and is commonly used in cosmetics and confectioneries as a solvent, plasticizer, humectant, and sweetener. G can absorb up to 50% of its weight of water vapor from air and is evaporated easily (*17*). Thus, the plasticizing effect of S is known to last longer than G. S occurs widely in nature in most fruits, such as black grapes, apples, plums, and cherries, but is produced commercially by the hydrogenation of glucose. S is used in processed foods, confectioneries, toothpaste, and

other personal care products as a stabilizer, plasticizer, humectant, emulsifier, and bodying agent (*18*).

Film Formation. The WPI/plasticizer ratio was held constant to eliminate variations in the plasticizing effect, and the WPI/G = 2.1 and WPI/S = 1.2 ratios were applied to investigate films with similar mechanical properties (after 2 days). The concentrations of WPI were held constant at 10.8 and 11.7% (w/w) WPI for films plasticized with G and S, respectively. These concentrations are the critical gel concentrations (c_g) determined in Anker et al. (*10, 11*). The c_g is the minimum concentration needed to form a gel with the actual film formation procedure. The concentration of WPI and S was based on the dry weight. The solutions were mixed for 1 h, adjusted to pH 7, degassed, and heated to 76.5 °C at a heating rate of ≈6 °C/min. The cast solutions were allowed to cool and dry at room temperature for ≈4 h and were then dried in a climate room at 23 °C and 50% RH for 16 h. To ensure that the films could be peeled intact from the casting surface, the Petri dish was covered with a hydrophobic surface of Teflon FEP film (Norton Performance Plastics Corp., Akron, OH). Solution (15.4 g) was applied to each Petri dish to minimize thickness variations. For further details on the film formation, see Anker et al. (*9*). The films were stored in a climate room at 23 °C and 50% RH. The film properties were measured after preconditioning in the climate chamber for 2 days and were then measured at regular intervals for up to 120 days.

Film Thickness. Before testing, the thickness of the films was measured by a digital micrometer (model IDC-112CB, Mitutoya Corp., Tokyo, Japan) at five positions along the rectangular strips for the mechanical properties and at the center and at four positions around the perimeter for the water vapor permeability (WVP). The mechanical properties and WVP were calculated using the average thickness of each film replicate.

Mechanical Properties. A texture analyzer (model TA-XT2, Stable Micro Systems, Godalming, U.K.) was used to determine mechanical properties at large deformations in accordance with ASTM D882-91 (*19*). The films were tested in a climate room at 23 °C and 50% RH. Initial grip separation and crosshead speed were set to 50 mm and 24 mm/min, respectively. The tested film strips were 80 mm long and 6 mm wide. Force and elongation were recorded during extension, and Young's modulus (E), stress at maximum force (σ_y), stress at break (σ_b), and strain at break (ϵ_b) were calculated. These properties derived from the tensile test are often also referred to in the literature as modulus of elasticity, yield strength, tensile strength, and elongation. Yield strength is the tensile stress at which the first sign of nonelastic deformation occurs. At least 28 replicates of each experimental point were evaluated.

Moisture Content (MC). MC was determined by drying in an oven (model T6060, Heraeus, Mölndal, Sweden) at 105 °C for 24 h. Small test specimens were cut and put on glass Petri dishes, and the weights before and after the oven-drying were recorded. MC was calculated as the percentage of weight loss based on the original weight, in accordance with ASTM D644-94 (*20*). At least 14 replicates of each experimental point were evaluated.

Water Vapor Permeability. WVP was determined according to ASTM E96-90 (*21*) and corrected for the stagnant air gap inside test cups for hydrophilic films using the WVP Correction Method (*22*). A climate room containing fans was used to test WVP, and the fan speed was set to achieve an air velocity of >152 m/min above the test cups to avoid stagnant air and to minimize mass transfer resistance above the films. The climate room was set at 23 °C and 50% RH. Distilled deionized water was placed in the bottom of the test cups to expose the film to a high RH inside the test cups. The surface of the films that had been dried in contact with the hydrophobic Teflon film faced the high RH. After the films were mounted, the whole assembly was weighed. Weighing (with an accuracy of 0.01 g) was repeated at 30-min intervals. Steady state was achieved in <2 h. Thereafter, the weight of the assembly was recorded every 2 h during the first 8 h and finally after 24 h. At least nine replicates of each experimental

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Table 2. T_g and MC of WPI/G = 2.1 and WPI/S = 1.2 Films as a Function of Time

time (days)	WPI/G = 2.1		WPI/S = 1.2	
	MC[a] (%)	$T_g^{b,c}$ (°C)	MC[a] (%)	T_g^b (°C)
2	22.0 ± 0.4	−56 ± 3	9.1 ± 0.1	−14 ± 1
70	15.1 ± 0.6	−43 ± 1	8.9 ± 0.1	−11 ± 1
120	15.4 ± 0.6	−45 ± 1	9.4 ± 0.1	−15 ± 1

[a] Mean values ± 95% confidence interval. [b] Mean values ± standard deviation. [c] No grease applied.

point were evaluated. In all cases, a linear regression of the steady-state data yielded R^2 values >0.999. Due to the stagnant air gap inside the cups, the RH on the underside of the films varied between 72 and 80%. Consequently, the gradient varied between 50 and 72% and 50 and 80%. For further details on the cups and the WVP measurements, see Anker et al. (*9*).

Dynamic Mechanical Analysis. The small deformation analysis of the films was performed in tension in a Rheometrics solids analyzer, RSA-II (Rheometrics Scientific, Piscataway, NJ). The tested film strips were 35 mm long and 4 mm wide and were clamped in the instrument with the initial grip separation of 23.4 mm. The films were subjected to a sinusoidal strain on top of a static deformation, the sum of these being nondestructive. The magnitude and phase of the resulting sinusoidal stress were measured, together with the total elongation (ΔL) of the film strip during the measurements. The storage modulus (E') and the loss modulus (E'') were calculated together with the phase angle (δ); tan $\delta = E''/E'$. T_g was measured as the temperature of the tan δ peak. The testing was conducted at a constant frequency of 1 Hz, a strain of 0.01%, and over a temperature range of −65 to 100 °C, at a heating rate of 5 °C/min. The measurements of each experimental point were made at least in triplicates. The temperature sweeps were performed after the films were covered with hydrophobic grease. The method of covering the films with grease prevents moisture loss during temperature sweeps, which ensures correct measurements of the material properties (*23*). Increased ΔL values with increasing temperature were taken as the indicator of sufficient moisture retention.

RESULTS AND DISCUSSION

This work studied the aging of WPI films plasticized with G and S. Normally, a higher amount of S than G is needed to obtain similar tensile properties, and thus the WPI/G = 2.1 and WPI/S = 1.2 ratios were determined in order to investigate films with similar mechanical properties. The results from the WPI/G = 2.1 and WPI/S = 1.2 films are presented side by side in this study, first showing the result of the MC (Figure 1). The MC is important because of the plasticizing effect of water and thus the effect on the mechanical and barrier properties. The film thickness is then presented (Figure 2). A change in the film thickness may be indicative of a change in network structure and is hence presented at an early stage. Third, the mechanical properties are shown (Figures 3−5), and, fourth, the barrier properties represented by the WVP are presented (Figure 6). Finally, the results of the dynamic mechanical analysis, and thereby the T_g, are shown (Table 2).

Moisture Content. The MC of the WPI/G = 2.1 and WPI/S = 1.2 films is given in Figure 1. The MC of the WPI/G = 2.1 films decreased from 22% (2 days) to 15% (45 days) and was thereafter relatively constant at 15% (for up to 120 days), whereas the MC values of the WPI/S = 1.2 films were unchanged at ∼9%. These results show that WPI films plasticized with S are more stable when stored at constant temperature and RH. Both G and S most readily bind to water due to their
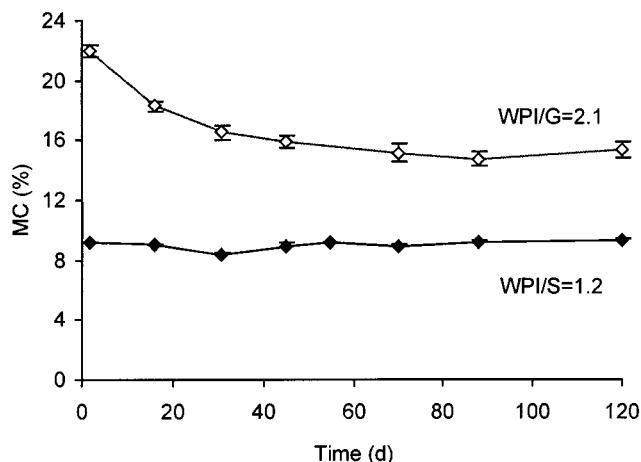

Figure 1. MC of WPI/G = 2.1 and WPI/S = 1.2 films as a function of time. Error bars show ±95% confidence interval.

hygroscopic character, and this can be exemplified by the use of both plasticizers as moisture conditioner in a variety of products such as moisturizing creams, toothpaste, and pharmaceuticals (*18*). G, however, has a higher molecular mobility owing to its lower molecular weight (G = 92 Da; S = 182 Da) and thus migrates more easily. Consequently, the decreased MC for the WPI/G = 2.1 films indicates that a simultaneous migration of G occurred. This is in agreement with the findings of Adcock et al. (*8*), who stated that the presence of a second additive influences the migration of a first additive and that an additive of the same class migrates in similar ratios. This is applicable in the present study, in which water and G were added as first and second additives, respectively. Accordingly, the MC of the WPI/S = 1.2 films is constant due to the higher molecular weight of S, which results in lower molecular mobility. Consequently, no migration of S occurred during the period of the study.

The higher MC of the WPI/G = 2.1 films is most probably due to the more hygroscopic character of G, which therefore has a higher affinity than S for binding water at equivalent RH and temperature (*15, 24*). The variation in MC is an important parameter, owing to the plasticizing effect of water on biopolymeric films (*25−31*).

The aging of the WPI/G = 2.1 films *was* detectable by visual observation because the films become rather fragile with time and the paper envelopes (in which the films were stored) wrinkled as a result of migrated plasticizers from the film matrix to the surface. This was not detected for the WPI/S films. Visual observations of WPI/S films stored for 6 months and up to 1 year show major crystallization of S on the surface of the films.

Film Thickness. The thickness of the WPI films as a function of time is shown in Figure 2. Both the WPI/G = 2.1 and WPI/S = 1.2 films have uniform film thickness. Even when the MC decreases for the WPI/G = 2.1 films, the thickness is not affected. Apparently, this decrease in MC is too small to result in any effect on the network structure, most probably due to the fixed protein matrix. More severe conditions, such as an increased RH, increase the MC and, consequently, the film thickness, due to the swelling of the protein matrix. This was shown in Anker et al. (*10*), where WPI/S = 1.2 films exposed at 23 °C and 82% RH for 48 h increased in thickness by 30%.
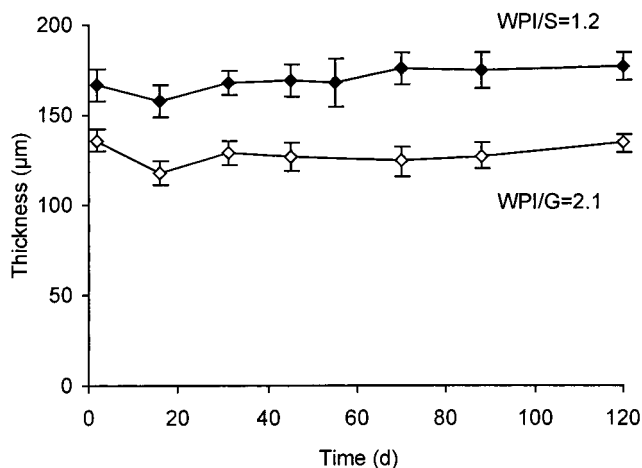
Figure 2. Film thickness of WPI/G = 2.1 and WPI/S = 1.2 films as a function of time. Error bars show ±95% confidence interval.
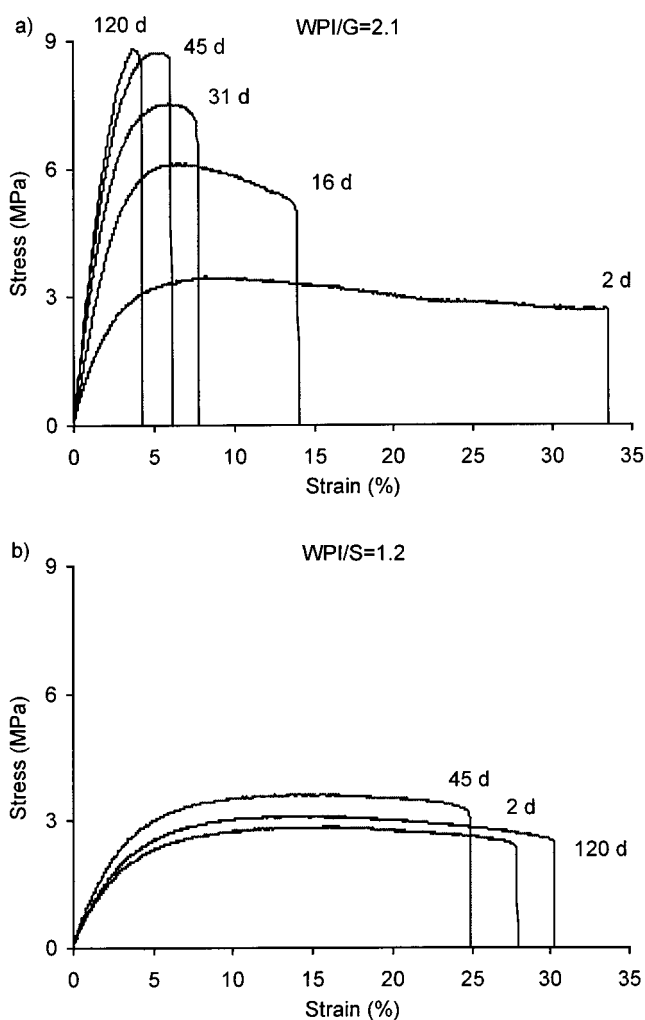

Figure 3. Representative stress and strain curves of (a) WPI/G = 2.1 and (b) WPI/S = 1.2 films as a function of time.

The thicker WPI/S = 1.2 films as compared with the WPI/G = 2.1 films are a result of the higher WPI concentration. The concentrations of WPI were 11.7 and 10.8% (w/w) for films plasticized with S and G, respectively. At higher concentrations, less water is present and, during the drying process of the films, water evaporates and thicker films are produced.
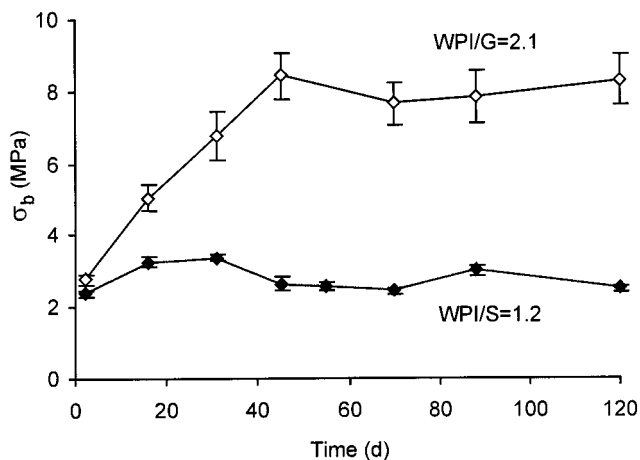

Figure 4. Stress at break (σ_b) of WPI/G = 2.1 and WPI/S = 1.2 films as a function of time. Error bars show ±95% confidence interval.
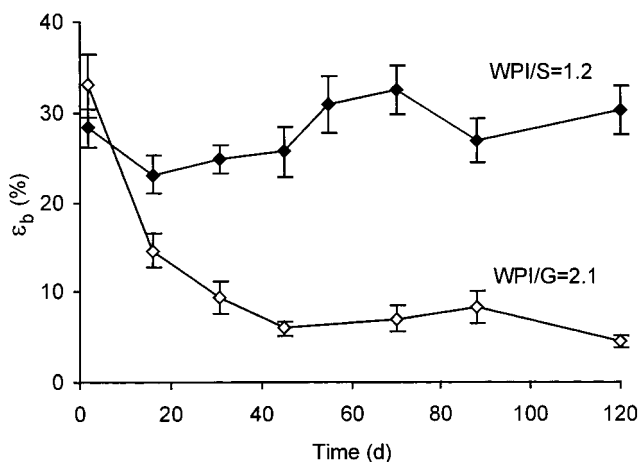

Figure 5. Strain at break (ϵ_b) of WPI/G = 2.1 and WPI/S = 1.2 films as a function of time. Error bars show ±95% confidence interval.

Mechanical Properties. Figure 3 shows the representative stress and strain curves of the WPI/G = 2.1 and WPI/S = 1.2 films. It is clearly visible that the WPI/G = 2.1 films become stiffer and less extendable with time, whereas the WPI/S = 1.2 films are more or less unaffected. These effects are more clearly visualized in Figures 4 and 5, where the stress at break (σ_b) and strain at break (ϵ_b) are plotted as a function of time. The behavior of Young's modulus (E) and stress at maximum force (σ_y) are almost identical with σ_b; thus, only the latter is presented in this paper. The σ_b increases rapidly from 2.8 to 8.4 MPa after 45 days for the WPI/G = 2.1 film and then levels off. Accordingly, the ϵ_b decreases from 33 to 8% during the same time, and the films then become rather fragile. After 120 days, the σ_b and ϵ_b values are 8.3 MPa and 4%, respectively. The WPI/S films, on the other hand, had a relatively constant σ_b of ~2.5 MPa and an ϵ_b of ~30% during the whole study.

This effect on the mechanical properties correlates very well with the behavior of the MC shown in Figure 1. After 45 days, the MC of the WPI/G = 2.1 films decreased from 22 to 15% and was constant thereafter at 15%. Accordingly, the constant MC of the WPI/S = 1.2 films at ~9% results in constant mechanical properties. The reduced MC of the WPI/G = 2.1 films is also indicative of the G migration previously discussed. The
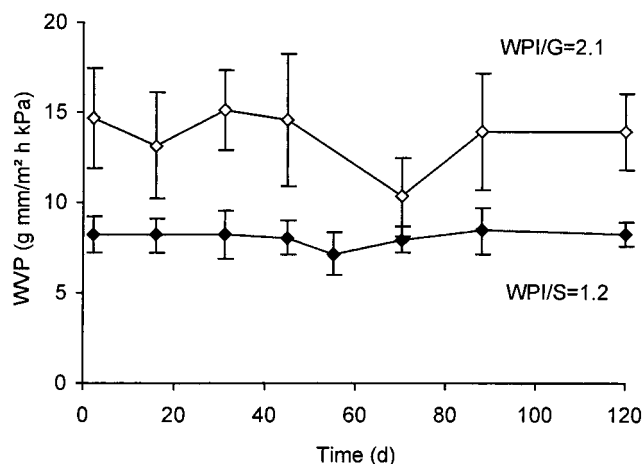
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Figure 6. WVP of WPI/G = 2.1 and WPI/S = 1.2 films as a function of time. Error bars show ±95% confidence interval.

migration of G and water makes the WPI/G = 2.1 films stiffer and less extendible. The leveling off after 45 days is most probably an effect of the ceased G migration. The variation in MC is an important parameter, due to the plasticizing effect of water on biopolymeric films (*25–27 31*).

Barrier properties. The WVP of the films are shown in Figure 6. It is clear that the WVP is relatively constant for both the WPI/G = 2.1 and WPI/S = 1.2 films during the time period of the study. This result in the WPI/G = 2.1 films is somewhat surprising because a rather large decrease in the MC has been found and the WVP generally decreases when the MC decreases. This may be explained by the microstructure of the films. Because the film thickness is intact, the protein matrix is intact and, thus, the pores in the matrix are most probably unaffected. In a previous study, the microstructure was related to the WVP, and it was shown that the WVP increased when the pore size increased for WPI films (*11*). Another explanation could be that the WPI/G = 2.1 film adsorbs water during the WVP measurements, which therefore results in unaffected WVP values.

A clear distinction is, however, that the barrier against water vapor is better in the WPI/S = 1.2 films than in the WPI/G = 2.1 films. This is due to the overall lower MC values of the WPI/S = 1.2 films and agrees with the findings of Maté and Krochta (*32*), who reported that WVP increases with increased MC for hydrophilic films as a result of the plasticizing effect of the water. Several researchers have reported increased WVP values when G instead of S was used as plasticizer (*11, 33, 34*). McHugh et al. (*34*) showed that WPI/G = 1 films exhibited significantly higher WVP values than WPI/S = 1.7 films. Chick and Ustunol (*33*) showed that casein-based films plasticized with G had higher WVP values than films plasticized with S when the same amounts of plasticizer were used.

Glass Transition Temperature (T_g). The T_g values are lower for the WPI/G = 2.1 films compared with those for the WPI/S = 1.2 films (Table 2). After 2 days, the WPI/G = 2.1 films and the WPI/S = 1.2 films have T_g values of −56 and −14 °C, respectively. This large difference in T_g values is due to the different physical properties of plasticizers. G is more hygroscopic, which imparts more water to the WPI/G films. A greater amount of water depresses the T_g significantly because water is the most abundant plasticizer for biopolymers.

The ability of a plasticizer to depress the T_g increases also with decreased molecular weight, and water has a very low molecular weight (*35*).

The T_g increases as the MC decreases for the WPI/G = 2.1 films, whereas the T_g is relatively constant for the WPI/S = 1.2 films. This is due to the unchanged MC values. The increase from −53 to −45 °C after 120 days for the WPI/G = 2.1 films, with the entire increase occurring after 70 days, is due to the relatively large decrease in the MC. Several researchers have shown increased T_g values when the MC decreased (*10, 25, 31, 36–39*). The reduced plasticizing effect of water leads to a decrease in the segmental mobility of chains in the amorphous regions of glassy and partially crystalline polymers and less structural relaxation at increased temperatures (*40, 41*).

Arvanitoyannis et al. (*37*) observed increased T_g values as the MC decreased for gelatin/soluble starch/water blends. This was explained by decreased hydrogen bonding between the starch–water chains and increased intermolecular hydrogen bonding between the starch chains. This leads to a higher interaction energy between the biopolymer chains and thus decreased segmental mobility. Arvanitoyannis et al. (*36*) showed a considerable impact on the T_g when the MC varied in blends with corn starch/sorbitol/water. T_g increased from −18.9 to −4.4 °C when the concentration of sorbitol decreased from 28 to 24% (w/w). The authors implied that the decrease in sorbitol concentration would allow less moisture uptake. This was shown by Anker et al. (*9*), in that decreasing the concentration of sorbitol indeed decreased the MC. It was implied that the six hydroxyl groups that sorbitol contains can readily bond with the water molecules and thus increase the water-holding capacity of WPI films.

In a previously study (*10*), it was shown that the T_g increased for the WPI/S = 1.2 films from −21 to −17 °C when the MC decreased from 9.8 to 9.4%. This is in agreement with the present study, in which the T_g for the WPI/S = 1.2 films increased from −14 to −11 °C when the MC decreased from 9.1 to 8.9%. These results show that the effect on the T_g is ∼10 °C/1% MC. The WPI/G = 2.1 films, on the other hand, show an increase of ∼1.5 °C/1% MC. This difference in effect on the T_g is due to the differences in MC between the films. At low MC, the T_g is more affected than at high MC. Gontard and Ring (*38*) showed that the T_g of wheat gluten films became rapidly depressed at low MC (5 °C/1% MC for 0–10% MC) and more slowly at higher MC (3.5 °C/1% MC for 10–30% MC). Kakivaya and Hoeve (*42*) presented similar results for elastin (15 °C/1% MC for low MC and 5 °C/1% MC for 10–20% MC). However, in general, the depression of T_g on biopolymers is ∼5–10 °C/1% water (*30*).

Conclusions. This study provided valuable information on the aging of WPI films. The WPI films were plasticized with G and S, and the film properties were measured during storage. The MC of the WPI/G films decreased, whereas the MC of the WPI/S films was constant. This affected the mechanical properties, and the WPI/G films became stiffer and less extendable, whereas the WPI/S films remained unaffected. These results show that WPI films plasticized with S are more stable when stored at constant temperature and RH. Both G and S are hygroscopic and consequently bind to water, but the lower molecular weight of G results in a higher molecular mobility. Thus, the decreased MC for

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the WPI/G films indicates that a migration of G simultaneously occurred with the water migration. The results show that the migration of low molecular weight additives, such as plasticizers, is an important factor to consider in the design of the appropriate film formula. This migration affects the mechanical properties, and this may decrease the protective function of the film.

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