Transglutaminase Cross-Linked Egg White Protein Films: Tensile Properties and Oxygen Permeability

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A methodology for preparing biodegradable films from egg white proteins was developed in this study. Film formation was based on the partial denaturation of egg white proteins by a preheating treatment at pH 10.5, followed by enzymatic polymerization of the proteins at 50 °C, pH 8.2, using a Ca^{2+}-independent microbial transglutaminase. SDS−PAGE confirmed the cross-linking of protein and showed that the polymerization reaction increased with increasing preheating temperature (from 60 to 80 °C) before the enzyme treatment. Films plasticized with higher glycerol content possessed higher equilibrium moisture content, indicating higher film hydrophilicity. The moisture sorption isotherms were well described by the GAB equation. Tensile properties of the films were dependent on relative humidity (RH) and glycerol content. Oxygen permeabilities of the films were low under low RH conditions but increased strongly as RH increased. Films with reduced glycerol content were better oxygen barriers but were more sensitive to RH variation.

Keywords: *Egg white proteins; edible films; tensile strength; oxygen permeability; transglutaminase*

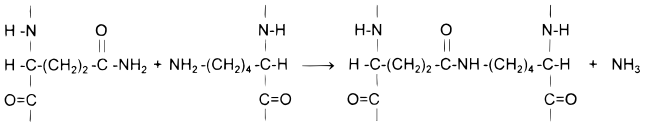
INTRODUCTION

Combined consumer demands for high-quality food products and reduced environmental consequences of packaging have generated an increased research interest on biodegradable films and coatings. The promise for such materials in food-packaging applications arises from their capability to supplement and possibly to improve the performance of existing synthetic packaging polymers, with reduced environmental impacts (Krochta and Mulder-Johnston, 1997).

A number of proteins, derived from both plant and animal origins, have been used as base materials for preparing biodegradable films, including casein, milk whey proteins, corn zein, wheat gluten, soy protein, gelatin, and others (Gontard et al., 1992; Mahmoud and Savello, 1992; Avena-Bustillos and Krochta, 1993; Park and Chinnan, 1995; Gennadios et al., 1996a; Arvanitoyannis et al., 1997). In general, barrier properties of protein films against oxygen and organic vapors are good under low relative humidity (RH) conditions but weaken greatly as RH is elevated. Protein films are poor water vapor barriers and sensitive to water due to the inherent hydrophilic nature of proteins.

Due to the high cohesive energy density of protein films (Miller and Krochta, 1997), plasticizers are often added to these polymers to improve their flexibility and stretchability. Plasticizers decrease the intermolecular forces acting along polymer chains, thereby imparting film flexibility, but at the same time weaken the film barrier against gases and vapors due to the enhanced segmental movement of the polymer chains. Moreover, plasticizers (mainly polyols) used with biopolymer films are hydrophilic and would likely alter the water sensitivity of the plasticized polymer. Therefore, integrated studies involving the evaluation of mechanical and barrier properties under various conditions of RH and plasticizer content are necessary to achieve films of desirable end-use performance.

Transglutaminase (TGase, protein-glutamine γ-glutamyltransferase, EC 2.3.2.13) is an enzyme capable of catalyzing acyl-transfer reactions, resulting in the formation of ϵ-(γ-glutaminyl)lysine intra- or intermolecular cross-links in proteins (Nielsen, 1995):



While flexible proteins such as bovine caseins are good substrates for this TGase catalyzed cross-linking reaction (Nio et al., 1985, 1986; Sakamoto et al., 1994), globular proteins such as β-lactoglobulin, α-lactalbumin, and bovine serum albumin are poor substrates due to their compact structures, which limit the accessibility of TGase to the target glutamine and lysine residues (Motoki and Nio, 1983; Mahmoud and Savello, 1992; Dickinson and Yamamoto, 1996; Matsumura et al., 1996). The susceptibility of these globular proteins to TGase can be enhanced by partially unfolding the proteins using various techniques, including treating whey proteins with dithiothreitol (Mahmoud and Savello, 1992; Færgemand et al., 1997a; Yildirim Hettiarachchy, 1997), partial denaturation of milk proteins at oil−water interfaces (Dickinson and Yamamoto, 1996; Chanyongvorakul et al., 1997; Færgemand et al., 1997b), and inducing a "molten globule state" in α-lactalbumin using ethylenediaminetetraacetic acid (Matsumura et al., 1996).

Egg yolk has a greater number of applications in the food industry compared to egg white, leading to a surplus of egg albumen in the egg-breaking industry of North America (Gennadios et al., 1996b). In an effort to develop further uses for this surplus product, we have chosen egg white as the base material for film prepara-

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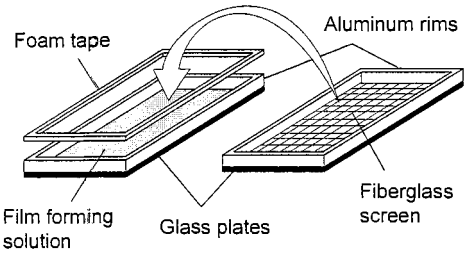

Figure 1. Casting trays for preparing TGase cross-linked egg white protein films. A fiberglass screen was attached to the lid for entrapping condensate droplets formed during the incubation of film-forming solution at 50 °C.

tion. The use of Ca^{2+}-independent TGase for polymerizing egg white protein to produce an edible film has not been studied to date, because it has been considered difficult to polymerize egg white proteins by TGase due to their compact structure. The objectives of this study were (1) to develop a methodology of utilizing hen egg white proteins to prepare biological polymer films using TGase as the cross-linking agent, (2) to investigate the effects of plasticizer (glycerol) content and RH on the mechanical properties of the films, and (3) to determine oxygen permeability coefficients of the egg white films ranging from 30 to 80% RH at 15, 25, and 35 °C.

MATERIALS AND METHODS

Materials. Egg white, separated from fresh hen eggs, was dialyzed against deionized distilled water for 2 days at 4 °C, freeze-dried, and stored in a freezer until use. Commercial grade microbial Ca^{2+}-independent TGase powder was purchased from Ajinomoto Co. Inc. (Tokyo, Japan) and partially purified before use. The powder was dispersed in deionized distilled water and centrifuged at 70000*g* for 1 h using a Beckman L8-M ultracentrifuge (Beckman Instruments, Inc., Spinco Division, Palo Alto, CA). The supernatant, containing 0.1% (w/w) TGase, was used for polymerizing the egg white proteins. Glycerol, potassium acetate, potassium carbonate, sodium chloride, sodium nitrite, lithium chloride, and potassium chloride were obtained from Fisher Scientific Ltd. (Nepean, ON).

Film Formation Method. An aqueous solution of freeze-dried egg white powder (4.4% w/w) was prepared and adjusted to pH 10.5 using 1 N NaOH. The resulting solution was heated at 80 °C for 20 min in a thermostated water bath, followed by the addition of glycerol [33−47% w/w, glycerol/(glycerol + protein)]. Undissolved aggregates were removed by filtration. Foams formed as a result of filtration were eliminated by applying and releasing vacuum to the solution repeatedly (six to seven times) until air bubbles disappeared. The egg white solution was then adjusted slowly to pH 8.2 using 0.1 N HCl before the enzyme was added (0.003% w/w). Sodium azide was added as a preservative (0.01% w/w).

The film-forming solution was spread on a leveled glass plate fitted with a rim around the edge. The plate surface was "polished" with a small quantity of high-vacuum silicone lubricant (Dow Corning Corp., Midland, MI) and then thoroughly wiped clean using ethanol. This treatment resulted in a glass surface that allowed the films to be easily peelable from the plate. The glass plate casting tray was covered with a lid (to prevent drying) and then incubated at 50 °C for 8 h to form a layer of translucent gel. Due to the high RH of the headspace above the gel-forming solution, condensate droplets tended to form on the inner side of the lid and drip into the gelling solution, resulting in films with uneven surfaces. To overcome this problem, a fiberglass screen was fitted underneath the glass lid to entrap the condensate droplets (Figure 1). Subsequent drying of the gel at the same temperature for 12−14 h with the lid removed resulted in transparent, colorless films.

Table 1. Equilibrium Relative Humidity Values for Selected Saturated Aqueous Salt Solutions at Various Temperatures (ASTM, 1985; Young, 1967)

	RH, %, at			
saturated solution	12.5 °C	22 °C	25 °C	35 °C
LiCl		11.3		
CH_3COOK	23.4	22.9	22.5	20.65
K_2CO_3	43.2	43.2	43.2	43.2
$NaNO_2$	67.0	65.0	64.4	62.3
NaCl	75.6	75.4	75.3	74.9
KCl	86.4		84.3	83.0

Electrophoretic Analysis of Polymerization. Sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was performed according to the method of Laemmli (1970) in a Mini-Protean II electrophoresis cell (Bio-Rad Laboratories Inc., Hercules, CA). Samples were run on 10% gels. Proteins were dissolved in sample buffer (100 mM Tris-HCl, pH 6.8) in the presence of β-mercaptoethanol, heated for 10 min at 95 °C, and loaded onto the gel at a concentration of 15 μg/well. Gels were run at a constant current (20 mA/slab gel). Gels were stained with Coomassie brilliant blue R-250 in 10% acetic acid/30% methanol.

Moisture Sorption. Moisture sorption isotherms of films containing 35 and 45% glycerol were determined gravimetrically under various RH conditions at 12.5, 25, and 35 °C. Heating has been shown to induce changes in the physical properties of protein films (Gennadios et al., 1996a; Miller et al., 1997). To avoid any curing effect that may arise due to the heating process, drying of the films was carried out in the presence of desiccant (Drierite, W. A. Hammond Drierite Co. Ltd., Xenia, OH) at 30 °C. Film samples were cut into small pieces and dried for 2 weeks and then equilibrated in glass bottles maintained at selected RH using appropriate saturated salt solutions (Table 1). Equilibrium moisture contents [EMC = (gain in mass/dry mass) × 100%] were taken when no further weight gain was observed in the samples.

Mechanical Testing. Ultimate tensile strength (*TS*) and elongation at break (*E*) of the films were determined using an Instron universal tester (Instron Corp., Canton, MA) equipped with pneumatic-action grips. Samples were cut into 15 mm wide and 80 mm long strips and equilibrated in glass chambers maintained at various RH and room temperature (22 °C) for 3 days before testing. Initial sample length and crosshead speed were 50 mm and 300 mm/min, respectively. Three thickness measurements were taken along each specimen with a micrometer (Mitutoyo Corp., Tokyo, Japan), and the mean values were taken for calculation. The typical film thickness was 0.10 ± 0.02 mm.

Effects of glycerol content and RH on the mechanical properties of the egg white films were investigated using response surface methodology based on the Central Composite Rotatable Design as described by Cochran and Cox (1992). The two-variable design codes were $-2^{0.5}$, -1, 0, 1, $2^{0.5}$. The coded and actual values of glycerol content and RH for the design are summarized in Table 2. The RH values of the selected saturated solutions (22 °C, Table 1) were approximated reasonably well with the chosen RH levels of the experimental design. The following second-degree polynomial model was used for fitting the *TS* and *E* values:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (1)$$

In eq 1, β_0, β_1, β_2, β_{11}, β_{22}, and β_{12} are the regression coefficients; X_1 and X_2 are the coded independent variables for glycerol content and RH, respectively. The GLM procedure from SAS (SAS Institute Inc., 1989) was used for the statistical analyses.

Oxygen Permeability Measurement. The oxygen transmission rate (OTR, mL m^{-2} day^{-1}) was determined using an Ox-Tran permeability tester (Mocon Inc., Minneapolis, MN). Films were placed in the test cell, where one side (upstream) of the film was exposed to oxygen and the other (downstream) to nitrogen carrier gas. To prevent the coulometric sensor from overloading, a 0.5% (v/v) oxygen in nitrogen mixture was used

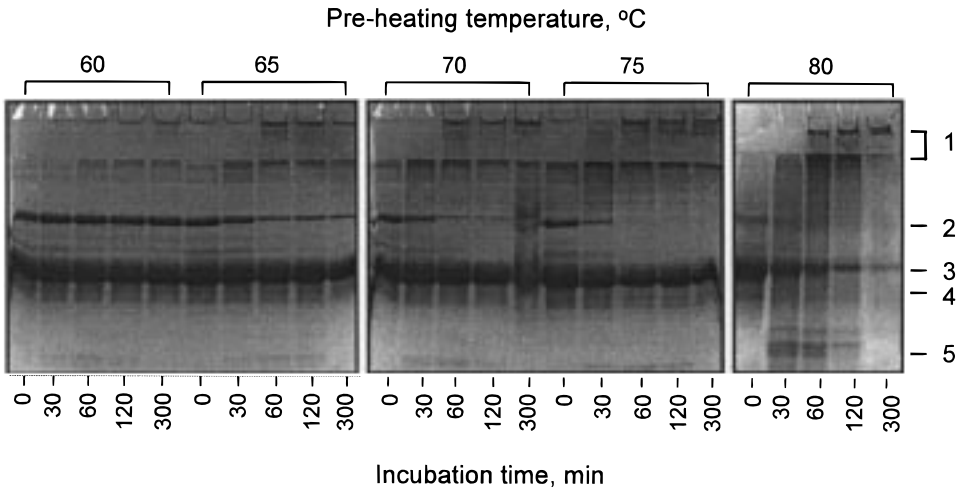

Figure 2. SDS−PAGE patterns of egg white proteins subjected to preheating treatments at various temperatures (pH 10.5 for 20 min), followed by incubation in the presence of TGase (pH 8.2, 50 °C): 1, polymerized protein; 2, ovotransferrin; 3, ovalbumin; 4, ovomucoid; 5, lysozyme.

Table 2. Responses of Dependent Variables for the Central Composite Rotatable Design

	independent variable		dependent variable	
design point	glycerol content,[a] %	RH,[a] %	TS,[b] MPa	elongation at break,[b] %
1	32.93 ($-2^{0.5}$)	43.4 (0)	5.96	58.5
2	35 (−1)	21.31(−1)	8.70	16.6
3	35 (−1)	65.49 (1)	2.01	75.4
4	40 (0)	12.17 ($-2^{0.5}$)	9.63	2.59
5	40 (0)	43.4 (0)	2.53	112.8
6	40 (0)	43.4 (0)	2.73	79.9
7	40 (0)	43.4 (0)	3.44	85.0
8	40 (0)	43.4 (0)	2.85	88.3
9	40 (0)	43.4 (0)	3.84	77.8
10	40 (0)	74.63 ($2^{0.5}$)	0.88	66.7
11	45 (1)	21.31 (−1)	3.19	77.1
12	45 (1)	65.49 (1)	0.79	75.6
13	47.07 ($2^{0.5}$)	43.4 (0)	1.48	100.5

[a] Values in parentheses are design codes for the independent variables. [b] Values are averages of at least 10 observations

as the upstream gas. Because the flow rate was low (fixed at 10 mL/min), the partial pressure difference (ΔP) across the films was essentially 0.005 atm (0.51 kPa). Both up- and downstream sides of the film were maintained at the predetermined RH. Testing was performed over a range of RH (≈30−≈80% RH) at three temperature levels (15, 25, and 35 °C). Oxygen permeability coefficients (mL μm m^{-2} day^{-1} kPa^{-1}) were calculated by multiplying OTR with the film thickness and dividing by the ΔP.

Rheological Measurement. Viscoelastic properties of the egg white protein solutions were investigated by small-amplitude oscillatory measurement using a Carri-Med CSL^2 500 rheometer (TA Instruments, New Castle, DE). The rheometer was equipped with coaxial cylinder fixtures, consisting of a fixed outer cup and rotating bob. Small-deformation controlled shear strain was applied to the sample in the linear viscoelastic regime (1% strain amplitude) at a constant frequency of 1 Hz. A constant temperature of 50 °C was maintained by circulating a thermostated fluid through the jacket surrounding the cup. To prevent evaporation of the gelling solution, the annular space above the exposed egg white protein solution was covered with a thin layer of vegetable oil.

RESULTS AND DISCUSSION

Cross-Linking of Proteins and Film Formation. It has been suggested that the formation of stable intermolecular β-sheet structure is the major change occurring in the thermal denaturation and aggregation of native egg white proteins (Mine et al., 1990). At pH values far from the pI and at low ionic strength, due to the electrostatic repulsion forces that hinder the formation of random aggregates, it is possible to thermally denature egg white proteins without forming coagula (Doi et al., 1994). Under these conditions, the proteins are partially unfolded and more flexible than when in their native form (Mine et al., 1990). Proteins in this state are thought to be more susceptible to TGase attack (Matsumura et al., 1996). In Figure 2, SDS−PAGE patterns of egg white proteins are shown as functions of incubation time for various preheating temperature treatments. In contrast to β-lactoglobulin, which can be cross-linked by TGase under elevated pH conditions (pH 8.5−9.0; Færgemand et al., 1997a), evidence from the gel patterns showed that high pH alone would not induce sufficient conformational changes for the TGase cross-linking reaction to take place. As shown, only minimal amounts of aggregates were detected when samples were preheated at 60 °C. As the preheating temperature was increased to 80 °C, the aggregate bands progressively became more intensified. The formation of aggregates was accompanied by reductions in band intensities of the constituent proteins for the egg white, namely, ovalbumin, ovomucoid, lysozyme, and ovotransferrin. This provides evidence for the formation of higher molecular weight polymers through intermolecular cross-linking.

The cross-linking reaction was also supported by the oscillatory rheological measurements. The protein solutions exhibited near-zero storage moduli (G', indicative of gel elasticity) during the onset of TGase treatment but progressively increased with incubation time and eventually leveled off to maximal G' values (Figure 3). In addition, the rate of development of the gel rigidity increased with increasing TGase concentrations. These observations suggested that the development of more solidlike three-dimensional networks during the incubation treatment resulted from the formation of ϵ-(γ-glutaminyl)lysine cross-links in the proteins. Because the rate of gelation was dependent on TGase concentration, the increase in gel elasticity would be attributed to the formation of the covalent cross-links rather than the incubation treatment effects.

Qualitatively, without the addition of TGase, egg white protein solutions remained in a liquid state even

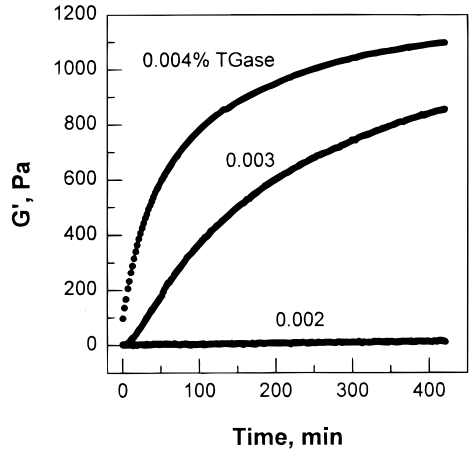

Figure 3. Effects of TGase concentration on the development of elasticity of egg white protein gels at 50 °C.

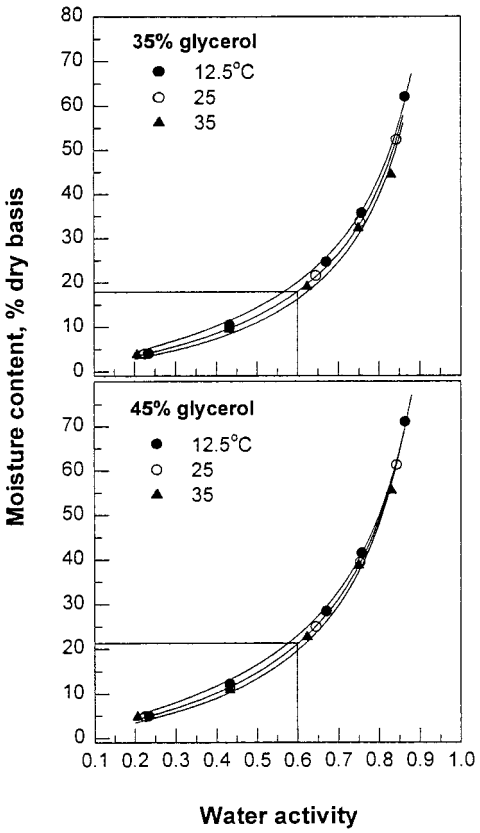

Figure 4. Moisture sorption isotherms for TGase cross-linked egg white films, as affected by temperature and glycerol content. The solid lines were derived from the GAB equation (eq 2). The values of the GAB parameters, estimated from the nonlinear regression for the 35% glycerol content films, are $M_m = 12.8\%$, $C_0 = 0.000544$, $A_0 = 1.031$, $\Delta H_c = 19000$ J/mol, and $\Delta H_A = -210$ J/mol and for the 45% glycerol film are $M_m = 14.573\%$, $C_0 = 0.00144$, $A_0 = 1.156$, $\Delta H_c = 16700$ J/mol, and $\Delta H_A = -477$J/mol.

after prolonged periods of incubation. Subsequent drying of the solution resulted in films that cracked readily upon peeling from the casting tray. In contrast, films produced from protein solutions that went through the gelation process were strong and showed good integrity.

Moisture Sorption Behavior. EMC of films was higher at low temperature and increased strongly as water activity (a_w) increased to unity (Figure 4). The exponential increase of EMC with increasing a_w indicated that water sorption in the polymer did not follow Henry's law; that is, the solubility of water in the polymer varied with the partial pressure of water vapor (Brown, 1992). The concomitant plasticization and swelling of the polymer matrix as the moisture content of the film increased, which provided more binding sites for water sorption, may have caused enhanced water loading at elevated a_w. The upward curvature of the isotherms may also suggest the formation of water clusters in the polymer matrix (Orofino et al., 1969; Brown, 1980; Starkweather, 1980) at higher water activities.

At any given a_w, EMC values were higher for samples containing higher glycerol levels. Because an equal amount of protein was used in the preparation of each film, the higher EMC observed can be attributed to the larger amount of glycerol incorporated into the polymer system. The increased water loading of the films is hypothesized to be a result of the additional polar −OH groups introduced by glycerol, resulting in films with higher hydrophilicity.

The moisture sorption isotherms of the TGase cross-linked films displayed the typical type I shape commonly observed in food products (Labuza, 1984). Due to the water-sensitive nature of the films, an accurate description of the water sorption data is important. The Guggenheim−Anderson−de Boer (GAB) equation, which has been shown to describe accurately the moisture sorption isotherms of many food products, was used to model the isotherms (Tsami et al., 1990; Kiranoudis et al., 1993; Lim et al., 1995):

$$M = \frac{M_m A C a_w}{(1 - A a_w)(1 - A a_w + A C a_w)} \quad (2)$$

In eq 2 constants C and A are temperature-dependent according to

$$C = C_0 \exp(\Delta H_C / RT) \quad (3)$$

$$A = A_0 \exp(\Delta H_A / RT) \quad (4)$$

In these equations, M_m (%) is the monolayer moisture content, T is the absolute temperature (K), and R is the universal gas constant (8.314 J/mol·K). ΔH_C (J/mol) and ΔH_A (J/mol) are the differences in heat of monolayer and free water to those of multilayer water, respectively. Because temperature is included as a variable in the model, the GAB equation can be used for describing the EMC data obtained at different temperatures. To reduce the uncertainties due to successive regressions, eqs 3 and 4 were substituted into eq 2, and the resulting five-parameter equation was fitted to the sorption data using a nonlinear regression procedure, PROC NLIN in SAS (SAS Institute Inc., 1989). The derived GAB equations were plotted as solid lines in Figure 4, showing the goodness of fit to the experimental data.

On the basis of the Clausius−Clapeyron equation, the net isosteric heat of sorption (ΔH_{st}), defined as the total heat evolved during the sorption process at a fixed level of moisture content M minus the heat of condensation of free water, can be calculated according to (Fennema, 1985; Weisser, 1985; Kiranoudis et al., 1993):

$$\Delta H_{st} = -R\left(\frac{\partial(\ln a_w)}{\partial(1/T)}\right)_M \quad (5)$$

ΔH_{st} values, estimated from the derived GAB equations,

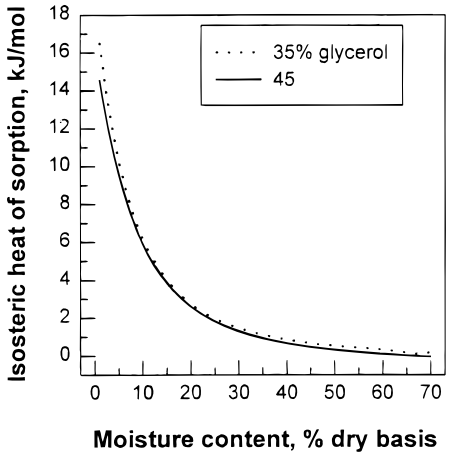

Figure 5. Net isosteric heat of sorption for TGase cross-linked egg white protein films. At a fixed moisture content (M), water activity (a_w) values at various temperatures were estimated from the GAB equation (eq 2).

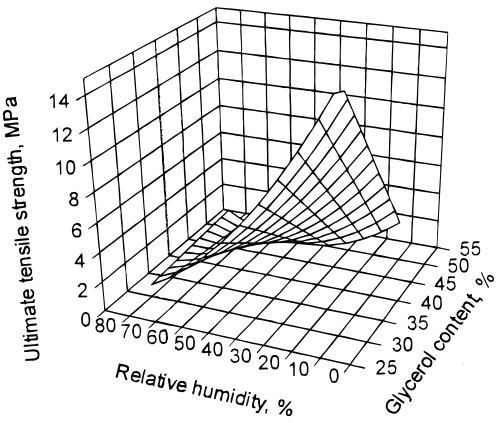

Figure 6. Ultimate TS as affected by RH and glycerol content [%, glycerol/(glycerol + protein)] of TGase cross-linked egg white protein films. The response surface was generated from eq 6.

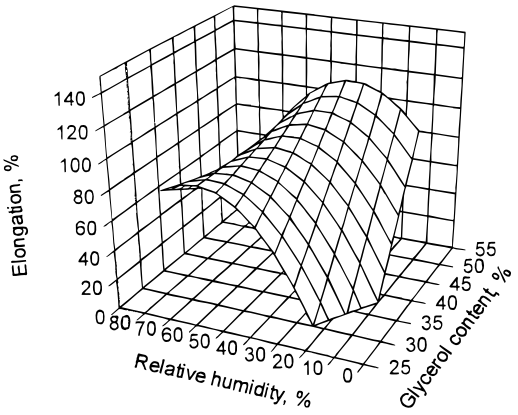

Figure 7. Elongation at break as affected by RH and glycerol content [%, glycerol/(glycerol + protein)] of TGase cross-linked egg white protein films. The response surface was generated from eq 7.

are summarized in Figure 5. As shown, the difference in affinity of water for both films was minimal, although the 35% glycerol samples tended to have higher ΔH_{st} values. This suggested that water molecules may have interacted more strongly with the lower glycerol content films. At higher moisture content levels, note that ΔH_{st} values approached zero. Therefore, the net heat evolved during the sorption process was essentially that of the heat of condensation for free water, implying that a large fraction of water present at high RH was highly mobile.

Mechanical Properties. Statistical analyses showed that β_{11} values in eq 1 for both TS and E were not significant ($p > 0.05$). Therefore, a reduced model with the X_1^2 term removed was used to fit the tensile properties. The resulting model described the experimental data well, with coefficients of determination (R^2) of 0.96 and 0.88 for TS and E, respectively. The best fitted regression equations take the forms

$$TS = 41.646 - 0.747X_1 - 0.663X_2 + 0.0018X_2^2 + 0.0097X_1X_2 \quad (6)$$

$$E = -381.16 + 8.926X_1 + 9.848X_2 - 0.043X_2^2 - 0.136X_1X_2 \quad (7)$$

where X_1 and X_2 are glycerol content of the film and RH, respectively.

The ultimate TS represents the maximal tensile stress the film could sustain before breaking, whereas the elongation at break reflects the extensibility of the material. Relationships between the independent and dependent variables are presented as three-dimensional response surface plots (Figures 6 and 7) generated from eqs 6 and 7. As shown, increasing glycerol content and RH strongly decreased the TS of the film, indicating that both water and glycerol were capable of plasticizing the cross-linked egg white protein films. TS was higher and more sensitive to RH variation when the plasticizer content of the film was low but progressively became weaker and less sensitive to RH as glycerol content was increased to higher levels. Note that above 70% RH, due to the extensive plasticization effect from the sorbed water, film samples were weak (TS < 2 MPa) with minimal change in TS regardless of the amount of glycerol used. The TS of the polymer is considered to arise from the interaction (primarily hydrogen bonding) between polymer molecules at various active sites along the chains. Plasticizers reduce the number of such polymer−polymer interactions by solvating the polar active sites, resulting in a lower cohesive energy density and therefore weaker physical strength (Paton, 1972; Meier, 1990).

Plasticizers can also be envisioned as "lubricants" that exist between the polymer chains, allowing the neighboring molecules to slide past each other more readily, thus increasing the extensibility of the polymer. This effect can be seen in Figure 7; in general, films with higher glycerol content possessed high E values. Below intermediate RH values, E increased strongly with increasing glycerol content. Maximal elongation, however, was observed near 50% RH. Above this RH, E dropped slightly and became less influenced by the change in glycerol content.

A stand-alone film should possess not only a reasonably high TS but also moderate elongation to impart flexibility. Although stronger films would be expected when lower amounts of glycerol are used (Figure 6), the corresponding films would possess very poor flexibility (Figure 7). Under these conditions, samples tended to crack or break when subjected to stress. In view of the water-sensitive nature of the films, RH conditions during the end-use application should be considered.

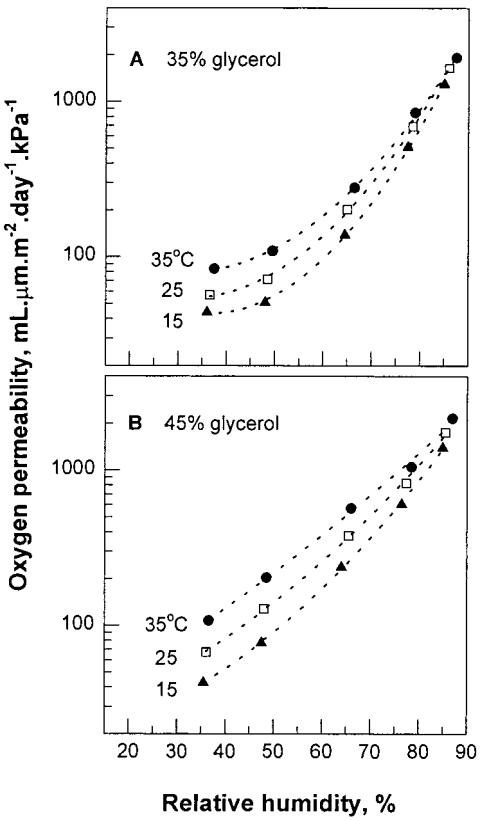

Figure 8. Oxygen permeability coefficients for TGase cross-linked egg white protein films with 35 and 45% glycerol content, as related to RH and temperature (symbols represent means of two repeated tests).

Oxygen Permeability. As with many reported protein-based edible films (McHugh and Krochta, 1994; McHugh et al., 1994; Gontard et al., 1996; Krochta and Mulder-Johnston, 1997; Miller and Krochta, 1997), transport properties of O_2 in the TGase cross-linked egg white protein films were RH-dependent (Figure 8). O_2 permeability coefficients for both films increased strongly with increasing RH (note that the plots are on logarithmic ordinates), and in general, higher permeability values were observed for the 45% glycerol films.

The strong function of O_2 permeability with RH could be related to the increase in diffusivity of the permeant, resulting from structural plasticization of the protein matrix caused by the sorbed water. Water is believed to increase the free volume of the polymer system and cause a drop in the polymer glass transition temperature (T_g), the temperature above which the macromolecules would possess sufficient energy to allow vibrational motion and undergo a glassy-to-rubbery state transition (Tager, 1978). In activated diffusion, this increase in polymer segmental movement would cause an increase in the diffusivity coefficient (D) of the permeant molecules. Permeation is governed by two processes: the thermodynamic dissolution of a permeant in the matrix and the kinetic diffusion of permeant through the matrix; that is, $P = DS$, where S is the solubility coefficient (Rogers, 1965; Robertson, 1993). As RH increased, the increased water content and formation of larger water clusters may also favor the solubility of oxygen (Gavara and Hernandez, 1994; Gontard et al., 1996), resulting in an overall increase in O_2 permeability.

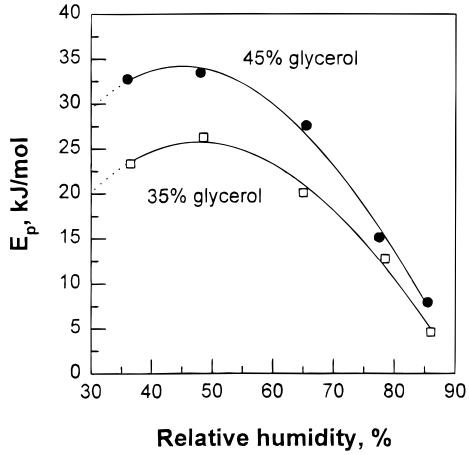

Figure 9. Apparent activation energy for oxygen permeation (E_p) for TGase cross-linked egg white protein films with 35 and 45% glycerol content, as affected by RH (symbols represent means of two repeated tests).

It is noteworthy that below ~50% RH, O_2 permeability was less sensitive to RH variation for 35% glycerol films as compared to their higher glycerol counterparts but increased more rapidly as RH increased beyond 50% RH (as evidenced by the greater slopes of the plots in contrast to the higher glycerol films). The permeability values eventually became similar for the two film compositions as RH approached saturation (Figure 8B). The prominent changes in slope of the permeability versus RH plots for the lower glycerol samples strongly suggest that the permeation of O_2 below and above the 50% RH break point was governed by different mechanisms. According to the free volume theory, the magnitude of T_g depression would depend on the volume fraction of the plasticizer in the polymer (Cowie, 1991). Below 50% RH, with the lower amounts of water sorbed, the polymer is assumed to exist in a glassy state, that is, the test temperature $< T_g$. The limited segmental movements of the polymer would reduce the influence of RH on the O_2 permeability. Accordingly, if the test temperature is elevated, the increased segmental motion would be expected to favor diffusion. This evidence can be seen in Figure 8A, where the change in slope of permeability versus RH plots became less prominent as temperature was increased. With the same argument, it can be concluded that the less conspicuous changes in slope for the 45% glycerol samples were due to the existence of the polymer in the rubbery state ($T > T_g$), as a consequence of more extensive plasticization due to the additional glycerol present.

The temperature dependence of permeability (P) can be described according to the classical Arrhenius equation

$$P = P_0 \exp^{-E_p/RT} \tag{8}$$

where P_0 is the preexponential constant. E_p is the apparent activation energy for permeation, which represents the summation of enthalpy of solution and activation energy of diffusion (Rogers, 1965; Robertson, 1993). As shown in Figure 9, the activation energies estimated for 35% glycerol films were lower than for 45% glycerol samples, especially in the low RH range. This implies that the O_2 permeability of the former was less sensitive to temperature changes. Over the low-

RH range, the increase in E_p as RH increased can be associated with the increasing molecular movements as temperature increased (Brown, 1992). As the polymer continued to gain moisture, the concomitant large-scale molecular motion and swelling of the polymer matrix cause a diminished resistance to the relatively small O_2 molecules; thus, a further increase in temperature would have minimal effect on O_2 permeability (Costello and Koros, 1994).

Conclusions. The high-pH thermal treatment proved to be effective for enhancing the susceptibility of egg white proteins to the cross-linking reaction catalyzed by TGase and may be considered for other globular proteins to achieve similar enzymatic polymerization. This method is more favorable than using dithiothreitol, which is not approved to be used in food applications. Egg white films with a spectrum of mechanical and oxygen barrier properties can be formed by varying the plasticizer content of the films. In view of the water-sensitive nature of the films, RH conditions during end-use applications should be taken into account during film fabrication. For instance, when barrier properties are of prime interest and the RH conditions are anticipated to be relatively high, low plasticizer content films should be used. In contrast, if mechanical properties are important and the RH conditions are expected to be low, a moderate amount of glycerol should be incorporated into the films.

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