

# Barrier and Tensile Properties of Transglutaminase Cross-linked Gelatin Films as Affected by Relative Humidity, Temperature, and Glycerol Content

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## ABSTRACT

Gelatin films were prepared by enzymatic cross-linking with transglutaminase, and their mechanical and barrier properties were evaluated as functions of relative humidity (RH, 30 to 75%), temperature (15 to 35°C) and glycerol content (15 to 31%). Water and glycerol plasticized the films synergistically, resulting in greater elongation but lower tensile strength values. Films with higher glycerol contents exhibited higher moisture contents, indicating higher hydrophilicity of the films. Permeabilities of oxygen and allyl isothiocyanate (an antimicrobial vapor from *Cruciferae* plants) were low when the films were dry, but increased considerably when RH>50%. Therefore, RH conditions during end-use applications must be considered to optimize the performance of these films.

**Key Words:** permeability, edible film, gelatin, relative humidity, tensile strength

## INTRODUCTION

BIOPOLYMER FILMS DERIVED FROM RENEWABLE SOURCES (proteins, carbohydrates and lipids) have gained considerable research interest. Such films may be used as food coatings or stand-alone film wraps to retard unwanted mass transfer in food products (Kester and Fennema, 1986; Miller and Krochta, 1997). They may also improve the recyclability of some packaging applications by reducing the need for complicated multilayer structures.

Although the use of compatible plasticizers is necessary to achieve flexible films, such additives affect film barrier properties (McHugh and Krochta, 1994). In general, protein films are excellent oxygen and aroma barriers. However, due to their inherent hydrophilic nature, such films tend to absorb large quantities of water at elevated relative humidity (RH) conditions, resulting in plasticized film matrices that have weakened barrier and mechanical properties. Therefore, knowledge on the effects of plasticizer and RH is essential for predicting the film performance during end-use applications.

Transglutaminase (TGase, protein-glutamine  $\gamma$ -glutamyltransferase, EC 2.3.2.13) catalyzes acyl-transfer reactions, resulting in the formation of  $\epsilon$ -( $\gamma$ -glutaminyl)lysine intra- and intermolecular cross-links in proteins (Nielsen, 1995). This enzyme has been demonstrated to be suitable for cross-linking various proteins to form biopolymer films. Motoki et al. (1987) reported that  $\alpha_{S1}$ -casein films polymerized by TGase were insoluble in water, 10% mercaptoethanol, 6.6M urea, 10% sodium dodecyl sulfate (SDS) and 6M guanidine hydrochloride. Mahmood and Savello (1992, 1993) cross-linked  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin using TGase, which resulted in films that resisted solubilization in aqueous buffers after various pH and heat treatments, but were digestible by proteolytic enzymes. Yildirim and Hettiarachchy (1998) utilized TGase to polymerize whey protein isolate, soybean 11S globulin, and a mixture of the two proteins to form biode-

gradable films. In these referenced studies, dithiothreitol (DTT) was used during film fabrication to render active sites of the proteins susceptible to the reactions with TGase.

In our study, gelatin was chosen as the film base material due to its random coil structure in solution, which was hypothesized to be readily polymerizable by TGase without the use of DTT. The use of DTT in food applications has been questionable due to its toxicity (Nielsen, 1995). The objective of this study was to evaluate the effects of RH, glycerol (plasticizer) content and temperature on the mechanical and barrier properties of gelatin films prepared using microbial  $\text{Ca}^{2+}$ -independent TGase as a cross-linking agent.

## MATERIALS & METHODS

### Materials

Gelatin (Type A, 275 bloom), was purchased from Fisher Scientific Ltd. (Nepean, ON). Commercial grade microbial  $\text{Ca}^{2+}$ -independent TGase powder (Ajinomoto Co. Inc., Tokyo, Japan) was partially purified before use by dispersing in deionized distilled water and centrifuging at  $70,000 \times g$  for 1 h using an ultracentrifuge (Model L8-M, Beckman Instruments Inc., Spinco Division, Palo Alto, CA). The supernatant, containing 0.1% (w/w) TGase, was used for polymerizing the gelatin protein. Reagents to be used for film preparation and testing (glycerol, potassium carbonate, sodium bromide, sodium chloride and sodium azide) were also from Fisher Scientific Ltd. Imidazole was from Sigma Chemical Co. (St. Louis, MO). Allyl isothiocyanate (AIT, >95% purity) to be used as a flavor volatile component in permeability tests, was from Aldrich Chemical Co. (Milwaukee, WI).

### Film formation

Films for mechanical testing ( $0.10 \pm 0.02$  mm thick) were prepared by dissolving various fractions of gelatin and glycerol in 25 mM imidazol-HCl buffer (pH 7) to give films with plasticizer contents ranging from 11.7 to 32.3% (w/w, glycerol/glycerol+gelatin). TGase and sodium azide were added at 0.005% (w/w) and 0.01% (w/w), respectively. The film forming solution was poured onto Plexiglas plates (fitted with rims around the edge, 30 cm  $\times$  17.5 cm) and allowed to react at 21°C for 4 h, followed by drying in an oven (50°C) for 12–14 h to produce transparent, colorless films. To shorten the testing time, thinner films of  $0.05 \pm 0.01$  mm were prepared for permeability tests.

### Electrophoretic analysis of polymerization

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970) in a Mini-Protein II Electrophoresis Cell (Bio-Rad Laboratories Inc., Hercules, CA). Samples were run on 7.5% separating gels. Proteins were dissolved in sample buffer (100 mM tris-HCl, pH 6.8) in the presence of 5% (v/v)  $\beta$ -mercaptoethanol, heated for 10 min at 95°C and loaded on the gel at a concentration of 15  $\mu\text{g}$ /well. The gel was stained with a Coomassie brilliant blue R-250 (Sigma Chemical Co., St. Louis, MO) in 10% acetic acid/30% methanol, and destained in a solution containing acetic acid/methanol/water (1:8:12, v/v/v). The gel

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was scanned on a Sharp JX-330 scanner (Sharp Electronics, Tokyo, Japan) and analyzed using an image analyzing software (Pharmacia ImageMaster 1D, Version 2.0, Pharmacia Biotech, Mississauga, ON).

### Moisture sorption

Moisture sorption isotherms of gelatin films with 15, 23 and 31% glycerol contents were determined gravimetrically at 15°C, 25°C and 35°C. In order to avoid possible curing effects that may arise due to heating (Gennadios et al., 1996a; Miller et al., 1997), film samples were dried at 30°C for 2 wk in hermetically sealed glass jars containing Drierite desiccant (W.A. Hammond Drierite Co. Ltd., Xenia, OH). The films were then equilibrated in glass jars maintained at selected RH using appropriate saturated salt solutions (ASTM, 1985). Equilibrium moisture contents ( $EMC = (\text{gain in mass/dry mass}) \times 100\%$ ) were taken when no further weight gain was observed.

The Guggenheim-Anderson-de Boer equation, which has been shown to describe isotherms of many food products accurately, was used to fit the moisture sorption data (Tsami et al., 1990; Kiranoudis et al., 1993; Lim et al., 1995):

$$M = (M_m A C a_w) / [(1 - A a_w)(1 - A a_w + A C a_w)] \quad (1)$$

where constants  $C$  and  $A$  are temperature-dependent according to:

$$C = C_0 \exp(\Delta H_C / RT) \quad (2)$$

$$A = A_0 \exp(\Delta H_A / RT) \quad (3)$$

In these equations,  $M_m$  (%) is the monolayer moisture content;  $T$  is the absolute temperature (K) and  $R$  is the gas constant (8.314 J/mol·K).  $\Delta H_C$  (J/mol) and  $\Delta H_A$  (J/mol) are the difference in enthalpy of monolayer and free water, compared to that of multilayer water, respectively. Since temperature is included as a variable in the model, the GAB equation can be used for organizing EMC data obtained at different temperatures. The GAB equation was fitted to the experimental data using SAS PROC NLIN (SAS Institute Inc., 1989).

### Tensile testing

Ultimate tensile strength (TS, force at failure/original cross sectional area) and elongation (E, increase in length/initial sample length, as %) at break of the films were measured using a Universal Tester (Model 1122, Instron Corp., Canton, MA) equipped with pneumatic-action grips (ASTM, 1990). Samples were cut into 15 mm × 80 mm long strips and equilibrated to selected RH as described. Initial sample length and crosshead speed were 50 mm and 300 mm/min, respectively. Three measurements of thickness were taken along each specimen with a micrometer (Mitutoyo Corp., Tokyo, Japan), and the mean values were used for calculations.

Response surface methodology was applied to evaluate the effects of glycerol content and RH on TS and E of the films. The levels of plasticizer content chosen were 11.7, 15, 23, 31 and 34.3% glycerol, and for relative humidity, 12.2, 21.3, 43.4, 65.5 and 74.6% RH. These levels corresponded to the design codes of  $-2^{0.5}$ ,  $-1$ ,  $0$ ,  $1$  and  $2^{0.5}$  for the rotatable central composite design as described by Cochran and Cox (1992). A second degree polynomial model was used for fitting the TS and E values:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (4)$$

where  $\beta_0$ ,  $\beta_1$ ,  $\beta_2$ ,  $\beta_{11}$ ,  $\beta_{22}$  and  $\beta_{12}$  are the regression constants;  $X_1$  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 statistical analyses.

### Oxygen permeability measurement

Oxygen transmission rate (OTR,  $\text{mL} \cdot \text{m}^{-2} \cdot \text{day}^{-1}$ ) was determined using an Ox-Tran 2/20 permeability tester (Mocon Inc., Minneapolis, MN). Samples were tested at 15°C, 25°C and 35°C, with both sides of the film maintained at predetermined RH levels. In order to prevent the

coulometric sensor from overloading, a 0.5% (v/v) oxygen in nitrogen gas mixture was used as the upstream gas. Since the flow rate was low (10 mL/min), the partial pressure difference ( $\Delta P$ ) across the films was essentially 0.005 atm (507 Pa). Oxygen permeability coefficients,  $P_{O_2}$  ( $\text{mL} \cdot \mu\text{m} \cdot \text{m}^{-2} \cdot \text{day}^{-1} \cdot \text{kPa}^{-1}$ ) were calculated by multiplying OTR by film thickness and dividing by  $\Delta P$ .

### Permeation of allyl isothiocyanate (AIT) and water vapors

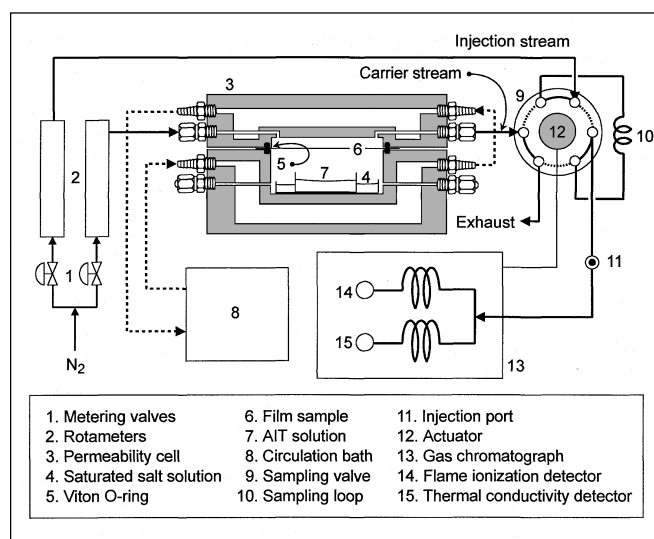
Permeabilities of water ( $P_{H_2O}$ ) and AIT ( $P_{AIT}$ ) vapors were determined using a permeability cell as described by Lim and Tung (1997). Briefly, the test film was mounted between two cylindrical half-cells to form upstream (high permeant concentration) and downstream (low permeant concentration) chambers (Fig. 1). A dish of AIT liquid was placed in the upstream chamber to provide AIT partial vapor pressures of 270, 530, 970 Pa at 15°C, 25°C and 35°C, respectively (Lim and Tung, 1997). Saturated solutions of  $K_2CO_3$ , NaBr and NaCl were used to provide constant RH conditions ranging from 43 to 75% RH (ASTM, 1985; Tagawa et al., 1993). Permeant concentrations in the downstream chamber were monitored by sampling 1.5 mL of the carrier stream and routing the gas sample to a gas chromatograph (GC, HP6890, Hewlett-Packard Co., Wilmington, DE) using a six-way sampling valve (Whitey Co., Highland Heights, OH). The valve was controlled by an actuator (Worcester Controls, West Boylston, MA) which was programmed to activate at predetermined time intervals by a 24 Volt DC signal from the GC.

AIT vapor in the upstream chamber was detected using a flame ionization detector (FID), while water vapor was detected by a thermal conductivity detector (TCD). In order to determine permeabilities of AIT and water vapors simultaneously, the injection stream was split and directed to the FID and TCD detectors via two separate columns (stainless steel 3.18 mm o.d., 60 cm long) packed with Porapak Q (Supelco Inc., Bellefonte, PA). Flow rates and temperature settings for the GC were:  $N_2$  carriers to FID and TCD, 15 mL/min;  $H_2$  to FID, 30 mL/min; air to FID, 240 mL/min;  $N_2$  reference to TCD, 45 mL/min; oven, 180°C (isothermal); TCD, 205°C; and FID, 260°C. Responses of the detectors were calibrated by injecting known amounts of AIT and water to the injection port using a gas-tight microsyringe (Hamilton Co., Reno, NV).

## RESULTS & DISCUSSION

### Mechanical testing of cross-linked gelatin films

SDS-PAGE patterns showed that gelatin treated with TGase re-



**Fig. 1**—Dynamic quasi-isostatic experimental arrangement for measuring AIT and water vapor permeabilities in TGase cross-linked gelatin films at various relative humidities.

**Table 1—Mechanical and oxygen barrier properties of TGase cross-linked gelatin films compared to other protein films**

Film <sup>a</sup>	Test conditions	Tensile strength (Mpa)	Elongation (%)	O <sub>2</sub> Permeability	Reference
Gelatin (Tgase)	—	Fig. 3	Fig. 4	Fig. 6	Present study
WPI:G (5.7:1)	23°C, 50% RH	29.1	4.1	18.5	McHugh and Krochta (1994)
WPI:G (2.3:1)	23°C, 50% RH	13.9	30.8	76.1	McHugh and Krochta (1994)
WPI:S (3.5:1)	23°C, 40% RH	—	—	0.7	McHugh and Krochta (1994)
WPI:S (3.5:1)	23°C, 70% RH	—	—	43.3	McHugh and Krochta (1994)
WG:G (5:1)	25°C, 0% RH	—	—	2.6	Gontard et al. (1996)
WG:G (5:1)	25°C, 95% RH	—	—	2726	Gontard et al. (1996)
Collagen	23°C, 63% RH	—	—	2.3	Lieberman and Gilbert (1973)
Collagen	23°C, 93% RH	—	—	89	Lieberman and Gilbert (1973)
SC:G (2:1)	23°C, 55% RH	2.98	29.89	—	Banerjee and Chen (1995)
WPC:G (2:1)	23°C, 55% RH	3.36	20.84	—	Banerjee and Chen (1995)
CZ:G (3:1)	25°C, 23% RH	19.5	—	—	Gennadios et al. (1993)
EggA:G (1:0.3)	25°C, 50% RH	4.12	12.4	—	Gennadios et al. (1996b)
EggA:G (1:0.5)	25°C, 50% RH	1.26	32.2	—	Gennadios et al. (1996b)

<sup>a</sup>Abbreviations: WPI=whey protein isolate; G=glycerol; S=sorbitol; WG=wheat gluten; SC=sodium caseinate; WPC=whey protein concentrate; CZ=corn zein; EggA=egg albumen. Units: mL·µm<sup>-2</sup>·day<sup>-1</sup>·kPa<sup>-1</sup>.

sulted in the formation of high molecular weight polymer aggregates that did not enter the stacking gel (Fig. 2, lanes 3 to 7). This was accompanied by a decrease in band intensities for the unpolymerized protein fractions. In contrast, no polymerization products were observed for the control (lane 2). These observations confirmed the formation of intermolecular cross-linked polymers catalyzed by TGase.

Statistical analyses of the tensile test data showed that β<sub>11</sub> for TS in Eq (4) was not significant (p>0.05). Therefore, a reduced model without the X<sub>1</sub><sup>2</sup> term was used to fit the TS. For E, all coefficients were significant (p<0.05). The resulting model described the experimental data well, with coefficients of determination (R<sup>2</sup>) of 0.97 and 0.92 for TS and E, respectively:

$$TS = 193.06 - 4.53 X_1 - 3.58 X_2 + 0.014 X_2^2 + 0.061 X_1 X_2 \quad (5)$$

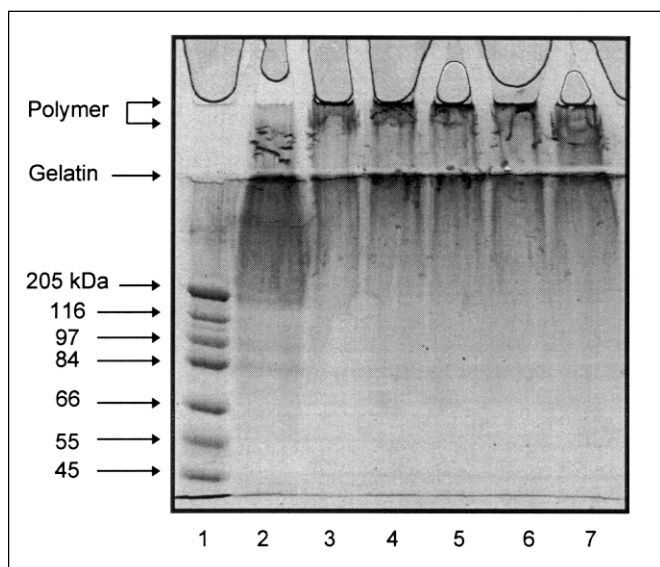
$$E = -1004.5 + 53.6 X_1 + 19.5 X_2 - 0.576 X_1^2 - 0.0848 X_2^2 - 0.337 X_1 X_2 \quad (6)$$

where X<sub>1</sub> represents the glycerol content and X<sub>2</sub> denotes the RH at which the film was equilibrated.

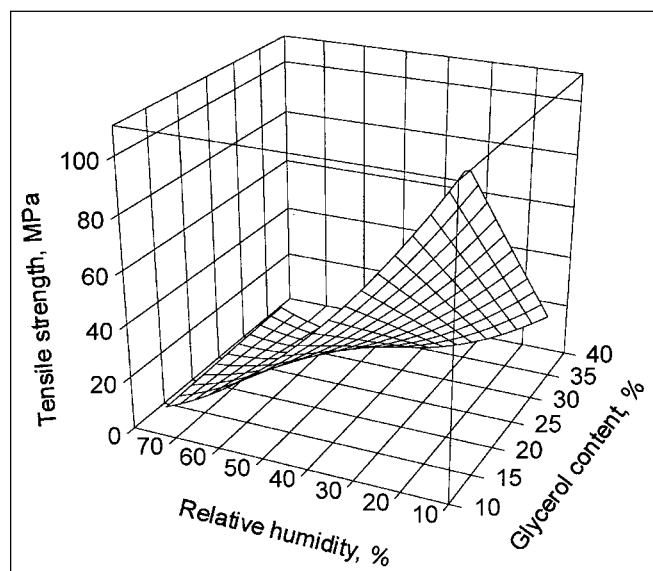
TS represents the maximal tensile force per original cross sectional area that the film could sustain before breaking, while E reflects the extensibility of the material. The effects of RH and glycerol content on TS and E can be seen on the response surface plots generated by Eq (5)

and (6) (Fig. 3 and 4). TS of gelatin films compared favorably with those of other reported protein films (Table 1). Although direct comparison between samples was unreliable due to varying test conditions and substrates used, the TGase cross-linked gelatin films appeared to have much higher elongation values, which may be attributed to the more deformable random-strand molecular structure of gelatin. Both water and glycerol were capable of plasticizing the polymer, thereby reducing TS values of the films (Fig. 3). Although films of lower glycerol contents had higher TS, the same samples were more sensitive to RH variation as compared to the higher glycerol samples. For instance, at 15% glycerol level, an increase of 15 to 75% RH caused a large drop in TS from 88 to 6 MPa; however, at 35% glycerol, a similar increase in RH resulted in a smaller change in TS from 16 to 7 MPa. This exemplified the plasticization effect of glycerol under low RH conditions. Note that above 70% RH, regardless of the amount of glycerol incorporated, TS values remained low (≈7 MPa) due to the extensive plasticization effect of water upon the polymer. In general, an opposite trend was observed for E; i.e., increasing RH and glycerol content resulted in films of greater stretchability (Fig. 4). Under combined high RH and high glycerol conditions, a slight decrease in E was observed. Excessively brittle samples were obtained when films of <20% glycerol content were exposed to low RH (<40% RH).

As demonstrated (Fig. 3 and 4), films of varying mechanical properties could be achieved by adjusting the plasticizer content. Stand-



**Fig. 2—SDS-PAGE patterns of gelatin treated with TGase. (1) molecular weight markers; (2) control; (3) 30 min; (4) 1h; (5) 2h; (6) 3h; (7) 5h.**



**Fig. 3—Ultimate tensile strength of TGase cross-linked gelatin films as affected by relative humidity and glycerol content at 22°C.**

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alone films should have not only good tensile strength, but also a reasonable elongation to impart flexibility. Although low-glycerol films had higher TS, the films were excessively brittle and susceptible to cracking when stressed, especially under low RH and low temperature conditions, where the biopolymers were in a glassy state. In view of the water-sensitive nature of the films, RH conditions during end-use applications should be considered during film fabrication.

### Moisture sorption isotherms

TGase cross-linked gelatin films exhibited J-shape moisture sorption isotherms (Fig. 5), suggesting the formation of water clusters in the polymer matrix as water activity increased. The strong function of EMC with water activity could be attributed to swelling of the biopolymer matrix, which may have caused an exposure of more water binding sites for water sorption. The derived GAB equation was plotted along with the experimental results (Fig. 5), showing its goodness of fit to the data.

In general, samples equilibrated at higher temperatures tended to

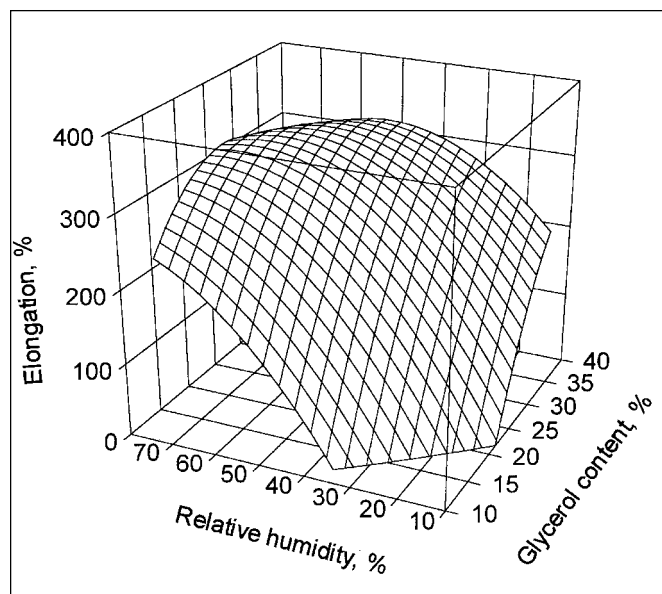


Fig. 4—Elongation at break of TGase cross-linked gelatin films as affected by relative humidity and glycerol content at 22°C.

have lower EMC values. The temperature effect, however, became less discernible at water activity  $>0.75$ . As water sorbed at high RH conditions was highly mobile, the diminishing temperature effect may be a result of the progressive domination of endothermic solubilization of glycerol by the bulk water over the exothermic moisture sorption process.

Note that at any given water activity, EMC values were higher for samples containing higher glycerol. This increased film hydrophilicity was hypothesized to be a result of the additional -OH groups introduced by glycerol. A similar observation had been reported by Mahmoud and Savello (1992) in TGase cross-linked  $\beta$ -lactoglobulin films.

### Oxygen permeation

$P_{O_2}$  values of the gelatin films were comparable to other protein films, and as with many biopolymeric films, oxygen permeation in TGase cross-linked gelatin films was highly RH-dependent (Fig. 6, Table 1). At any given RH conditions,  $P_{O_2}$  values were greater for films containing higher glycerol. During steady state permeation, the permeability coefficient ( $P$ ) of a film can be considered as two components represented by  $D$ , the diffusion coefficient, and  $S$ , the solubility coefficient, such that  $P=DS$  (Crank, 1975). The higher  $P_{O_2}$  values could be related to the increased diffusivity of oxygen in the polymer due to the plasticization effects of water and glycerol.

Large increases in  $P_{O_2}$  were noticeable above 50% RH (Fig. 6 insets), particularly for lower glycerol content films, such that the slopes of  $\log(P_{O_2})$  vs RH plots were appreciably greater when RH was raised above this intermediate region. According to the free volume theory, the magnitude of the glass transition temperature ( $T_g$ ) depression would depend upon the volume fraction of the plasticizer in the polymer (Cowie, 1991). At low RH, the polymer was hypothesized to exist in a glassy state, in which molecular segmental motions were restricted. As RH increased, hydrogen bonds operating between adjacent molecules were likely subjected to disruption due to the uptake of water (Stuart, 1994), causing a drop in  $T_g$  below the test temperatures, i.e., with the polymer in a rubbery-state. The stronger function of permeability with RH above intermediate RH values may thus be attributed to the enhanced oxygen diffusivity above  $T_g$  as a result of the  $T_g$  depression effect of water. Based on similar logic, if the polymer was plasticized sufficiently by glycerol and if the test temperature was higher than  $T_g$ , the change in slope of  $\log(P_{O_2})$  would become less discernible. The polymer would exist in the rubbery-state throughout the tested RH range. Evidence for these effects can be seen in the 31% glycerol, 35°C plot (Fig. 6).

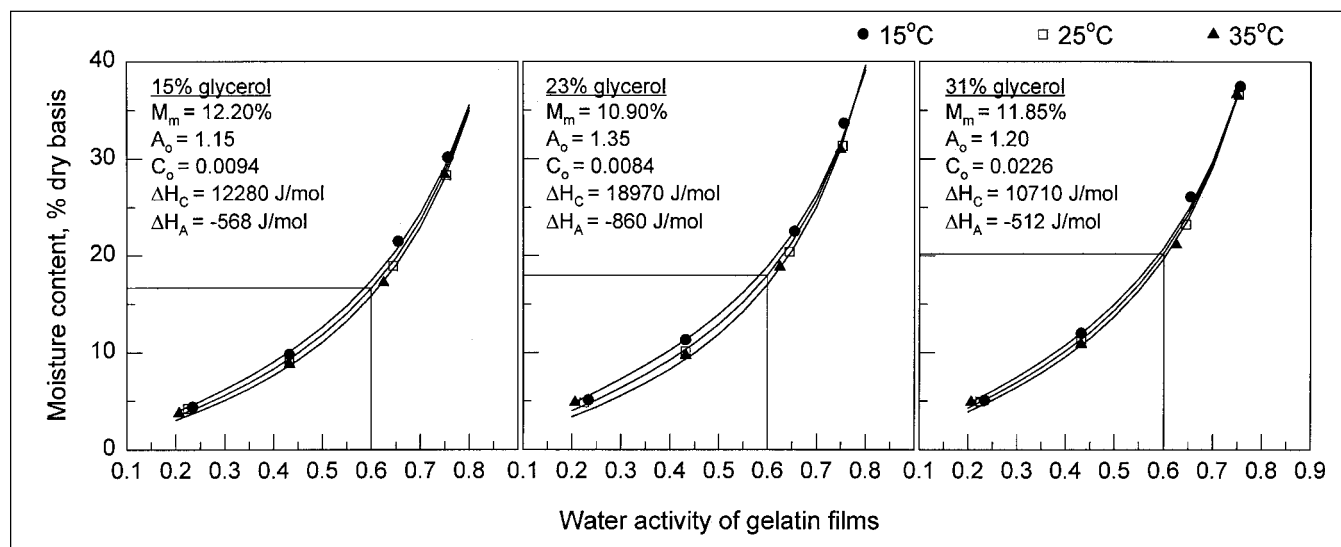


Fig. 5—Moisture sorption isotherms of TGase cross-linked gelatin films as affected by temperature and glycerol content. The solid lines were derived from the GAB equation (Eq 1).

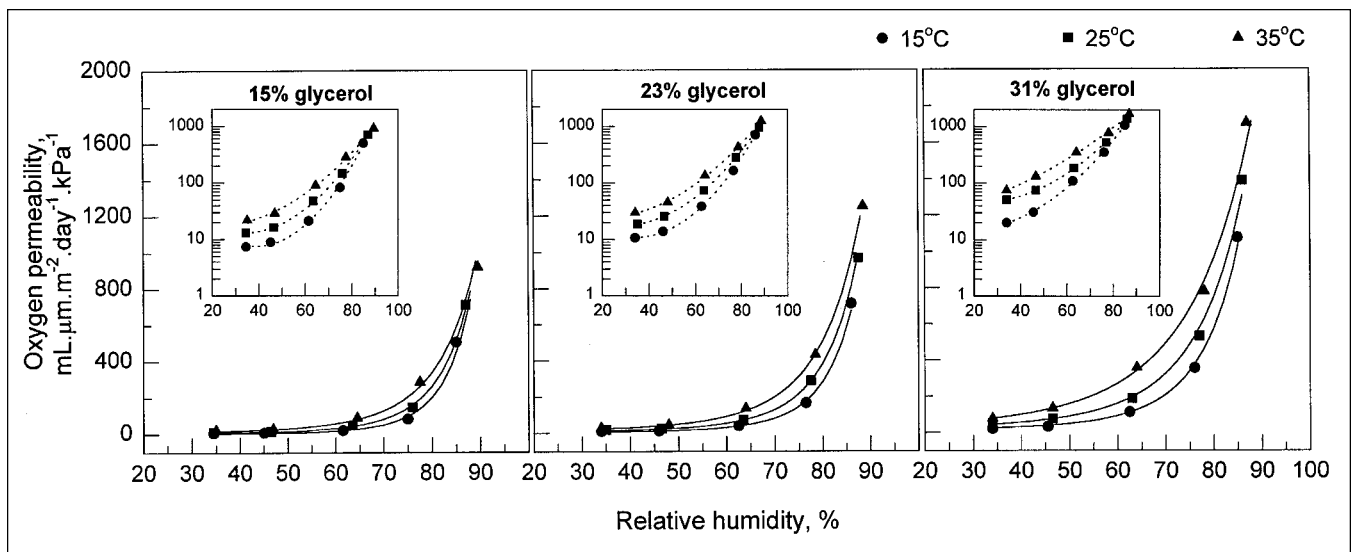


Fig. 6—Oxygen permeability in TGase cross-linked gelatin films as influenced by relative humidity, temperature and glycerol content. Insets show the same data plotted on logarithmic ordinates, revealing changes in slopes. Solid lines were derived from the least squares fits of Eq (8) to the data: 15% glycerol,  $\ln(P_{O_2})=3.05+0.81RH-0.0086RH^2-(261.51RH-2.91RH^2)/T$ ; 23% glycerol,  $\ln(P_{O_2})=3.13+0.71RH-0.0071RH^2-(226.87RH-2.43RH^2)/T$ ; 31% glycerol,  $\ln(P_{O_2})=3.47+0.88RH-0.0094RH^2-(267.65RH-2.99RH^2)/T$ .

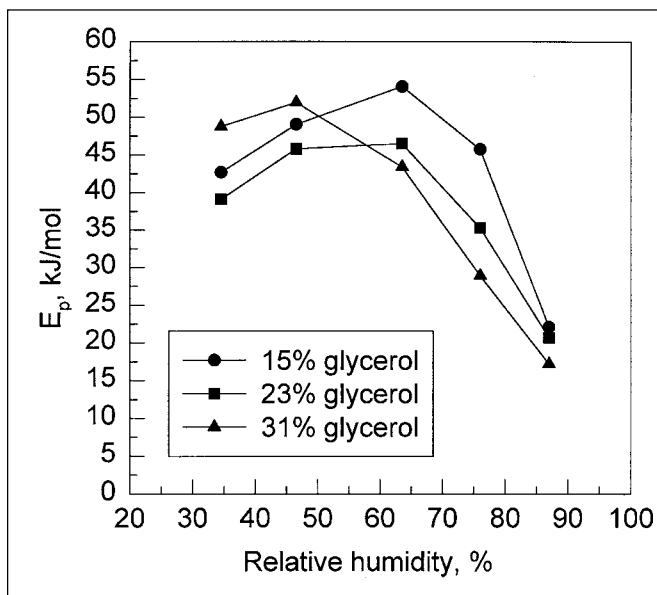


Fig. 7—Apparent activation energy for permeation of oxygen in TGase cross-linked gelatin films (15, 23 and 31% glycerol contents) as affected by relative humidity.

The apparent activation energy for permeation can be calculated according to the classical Arrhenius equation:

$$P = P_0 \exp(-E_p/RT) \quad (7)$$

where  $P_0$  is the pre-exponential constant.  $E_p$  is the apparent activation energy for permeation, which represents the summation of enthalpy of solution and activation energy of diffusion (Robertson, 1993). For all film types, maximal  $E_p$  values were observed (Fig. 7). Over the low RH range, the increased  $E_p$  with RH may be a consequence of increasing molecular movements arising from plasticization of the polymer matrix by water, causing a stronger function of oxygen permeability with temperature. As the polymer continued to gain moisture, the concomitant large-scale molecular motion and swelling of the film caused a diminished resistance to transport of the relatively small  $O_2$  molecules. Thus, a further increase in temperature would have minimal effect on  $O_2$  permeability (Costello and Koros, 1994).

Due to the strong function of  $P_{O_2}$  with RH above (50% RH, a simple exponential function was inadequate for fitting the permeabil-

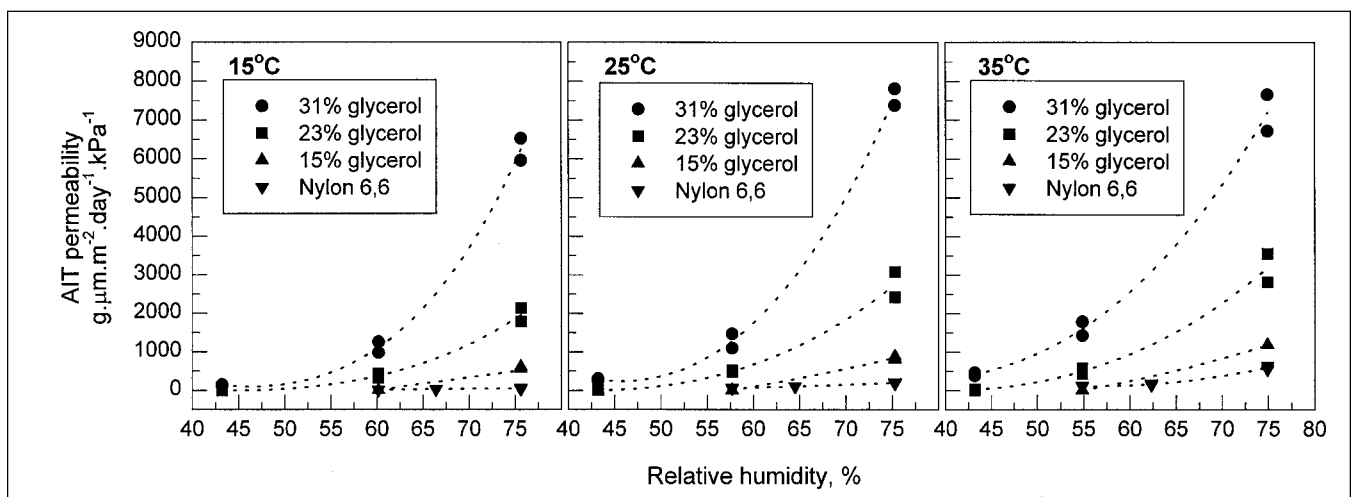


Fig. 8—AIT permeability in TGase cross-linked gelatin films as affected by relative humidity, temperature and glycerol content. Permeability data of nylon 6,6 film were included for comparison (Lim et al., 1998).

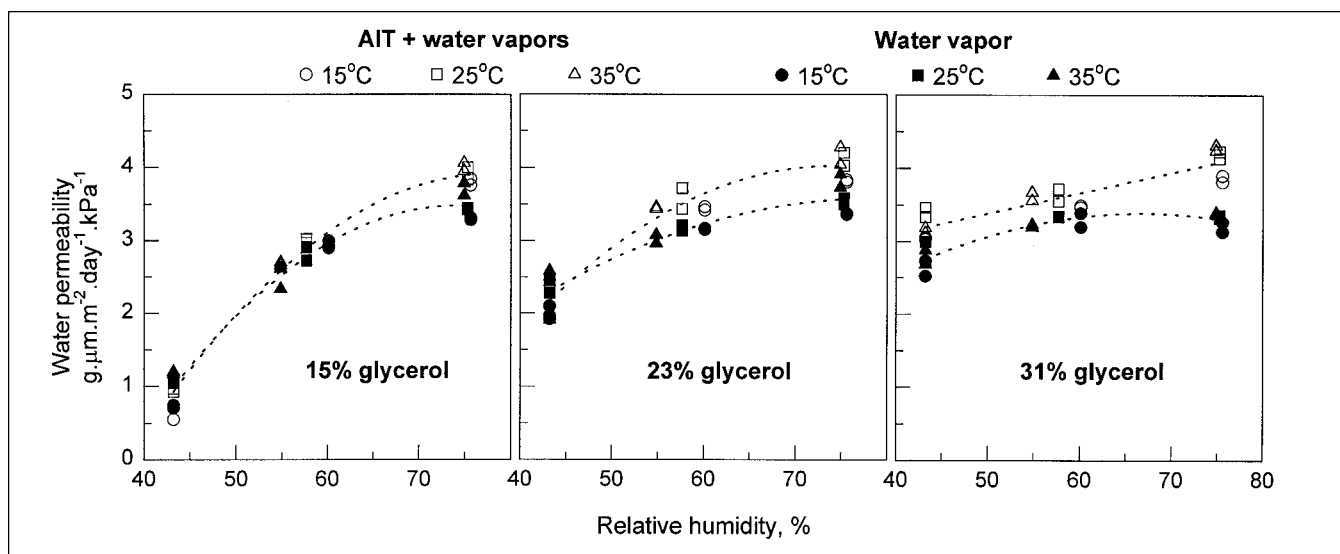


Fig. 9—Water vapor permeability in TGase cross-linked gelatin films as affected by glycerol content and AIT vapor.

ity data. Based on the Arrhenius equation, Eq (7), a two-variable equation was derived for correlating  $P_{O_2}$  with RH and temperature:

$$\ln(P_{O_2}) = (\alpha_0 + \alpha_1RH + \alpha_2RH^2) - (\beta_0 + \beta_1RH + \beta_2RH^2)(1/T) \quad (8)$$

where  $\alpha_{0,1,2}$  and  $\beta_{0,1,2}$  are regression constants. The constants were determined by fitting Eq (8) to the experimental data using the GLM procedure in SAS (SAS Institute Inc., 1989). The  $\beta_0$  terms were not significant ( $p > 0.05$ ) for all film types. Thus, a reduced model without  $\beta_0$  was used for fitting the data. The derived equation described the experimental data well (solid lines in Fig 6), with  $R^2$  values of 0.92, 0.95 and 0.98 for 15, 23 and 31% glycerol content films, respectively.

#### Permeation of AIT vapor

AIT ( $CH_2=CH-CH_2-N=C=S$ ), a natural aroma compound released by *Cruciferae* plants (e.g., horseradish and mustards) when their tissues are disrupted, has been shown to possess a wide antimicrobial spectrum (Isshiki et al., 1992; Mari et al., 1993; Tsunoda, 1994). In a review of isothiocyanates (including AIT), Delaquis and Mazza (1995) concluded that these compounds were promising for shelf-life extension of certain food products. For instance, AIT may be used as an antimicrobial vapor in modified atmosphere packaging applications.

Overall, transport properties of AIT were similar to those of oxygen, with  $P_{AIT}$  values being strong functions of RH (Fig. 8). In addition, water and glycerol acted synergistically to enhance the permeation of AIT in the polymer. This RH-dependent aroma transport in edible films had been reported by Miller et al. (1998) for whey protein isolate films, and by Debeaufort and Voilley (1994) for methylcellulose and gluten films. Also shown (Fig. 8) are  $P_{AIT}$  values for nylon 6,6 films for comparison. As shown, under low RH and/or low plasticizer conditions, AIT permeabilities of the cross-linked gelatin films were comparable to those of the nylon polymer, which is considered to be a good aroma barrier.

Given the low solubility of AIT in water (Weast, 1971), the strong function of AIT permeability with RH was probably caused predominantly by the enhanced penetrant diffusivity in the polymer. Activation energies for AIT permeation were higher at 43% RH (48.5 and 43.2 kJ/mol for 23 and 31% glycerol films) than at 75% RH (25.9, 17.8, 5.3 kJ/mol for 15, 23 and 31% glycerol films, respectively). The diminishing activation energies with RH, as hypothesized in the previous section, suggested an extensive swelling of the polymer.

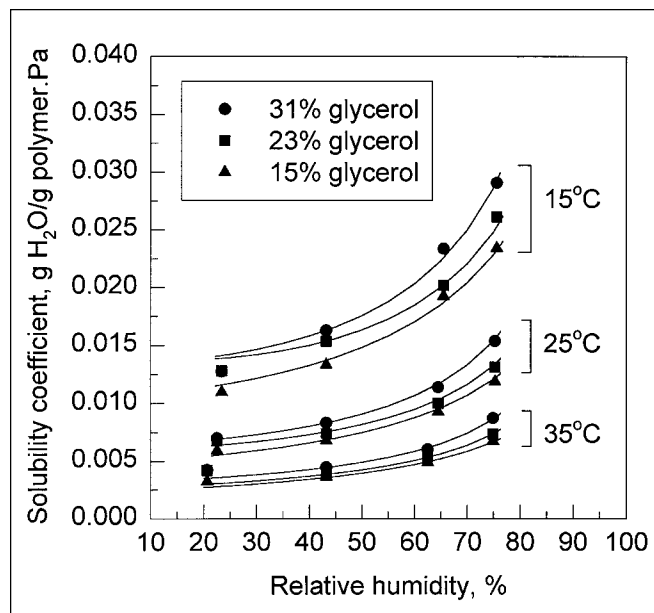


Fig. 10—Solubility coefficient of water in TGase cross-linked gelatin films as affected by relative humidity, temperature and glycerol contents.

#### Water vapor transport

$P_{H_2O}$  values increased with increasing RH for all films, with 15% glycerol film exhibiting the strongest influence of RH (Fig. 9). In addition,  $P_{H_2O}$  values were greater for films containing higher amounts of glycerol, especially when RH was low. This may be related to the higher hygroscopic nature of the films. Temperature appeared to have minimal effects on water vapor permeability. As shown (Fig. 10), the strong negative relationship of solubility coefficient with temperature indicated that the sorption process was exothermic. Although diffusivity was expected to increase with increasing temperature, the exothermic sorption process may have rendered the overall permeation less sensitive to the change in temperature.

Gelatin films exposed to AIT vapor provided higher  $P_{H_2O}$  values than those exposed to water vapor alone (Fig. 9). However, under low RH and low glycerol conditions, the effect of AIT vapor on water vapor transport appeared to be minimal. Since  $P_{AIT}$  values were low under the same conditions, this may suggest that the interactions of AIT with the polymer were limited when the polymer was dry. As the

film was further plasticized by water and/or glycerol, the hydrophobic moieties of the protein molecules became more accessible to AIT, which may have led to an increase in the intermolecular spacing of the polymer, causing the enhanced water vapor permeability.

**CONCLUSIONS**

TGASE CROSS-LINKED GELATIN FILMS OF VARYING MECHANICAL and barrier properties could be formed by adjusting the film glycerol content. Since RH interacted synergistically with glycerol content in plasticizing, and thus altering the performance of the films, RH conditions during end-use applications must be considered. Although the films exhibited good barrier properties under low RH conditions, their applications under higher RH environments would be limited due to their hydrophilicity. In order to reduce the moisture sensitivity and to maintain barrier integrity, hydrophobic layers (e.g., edible waxes and fatty acids) may be helpful if applied to film surfaces to shield the protein film from water. Further research in this area is needed to develop more versatile gelatin film structures.

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