

Comparison of Oxygen and Water Vapor Permeabilities of Whey Protein Isolate and β -Lactoglobulin Edible Films

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Oxygen permeability (OP) and water vapor permeability (WVP) of whey protein isolate (WPI) and β -lactoglobulin (β -Lg) edible films were studied at three different levels of glycerol content. WVP and OP of WPI films were not statistically different from WVP and OP of β -Lg films. Although the presence of glycerol as plasticizer could have masked some differences between β -Lg and WPI films, β -Lg appears to contribute to the barrier properties of the protein matrix of WPI in a manner similar to the other whey protein fractions. Temperature had an exponential effect on the OP of WPI and β -Lg films. Results fitted the Arrhenius model with activation energies in the 10.5–13.5 kcal/mol range.

Keywords: *Whey protein; β -lactoglobulin; edible films; oxygen permeability; water vapor permeability*

INTRODUCTION

Edible films and coatings, by acting as barriers to control the transfer of moisture, oxygen, carbon dioxide, lipids, and flavor compounds, can prevent quality deterioration and increase the shelf life of food products. Properties and potential uses of edible films and coatings based on polysaccharides, lipids, and protein materials have been reviewed (Kester and Fenemma, 1986; Guilbert, 1986; Krochta, 1992; Krochta et al., 1994; Guilbert et al., 1996).

Whey protein, a byproduct of the cheese industry, has excellent nutritional and functional properties and the potential to be used for edible films. The major fractions of whey protein are (Dybing and Smith, 1991) β -lactoglobulin (β -Lg, 57%), α -lactalbumin (α -La, 19%), bovine serum albumin (BSA, 7%), immunoglobulins (Igs, 13%), and specific polypeptides (PP, 4%).

Transglutaminase was used in the past to form films with β -Lg and α -La solutions, and the mechanical properties and water vapor transferability of such films were studied by Mahmoud and Savello (1992). However, the high cost of transglutaminase limits the practical use of these films. McHugh and Krochta (1994) developed a less expensive method to make whey protein films, based on heat denaturation of whey protein. They used whey protein isolate (WPI), a high-purity whey protein product (>95% protein). The effects of relative humidity (RH) and plasticizers on the water vapor permeability (WVP) and oxygen permeability (OP) of WPI-based films were examined (McHugh and Krochta, 1994; McHugh et al., 1994). Due to their hydrophilic nature, these films exhibit higher WVP and OP with increased RH. WVPs of these films were 3 orders

of magnitude higher than those of high-density polyethylene (HDPE) at high RH (McHugh et al., 1994). However, the OP is low and comparable to that of ethylene–vinyl alcohol copolymer (EVOH) at low or intermediate RH (McHugh and Krochta, 1994).

All whey protein fractions have globular conformations but different amino acid sequences and molecular weights (β -Lg, 18 300; α -La, 14 200; BSA, 66 300; Igs, 150 000–1 000 000; PP, 4 100–22 000). As a consequence, the conformations of the denatured proteins (unfolded) are different from each other. In addition, the number of disulfide bonds that influence the three-dimensional structure of protein varies considerably from one whey protein to another [β -Lg, 2; α -La, 4; BSA, 17; PP, none (Dybing and Smith, 1991)]. Intermolecular disulfide bonds are partly responsible for film structure. As a result, the molecular structure of a film made of denatured WPI is different from that of a film made of just one denatured whey protein fraction (e.g., β -Lg). These different structures could produce different permeability properties of the resulting films.

The objective of this work was to compare the WVP and OP of films made of WPI and β -Lg, the major fraction of WPI. By this comparison, the effect of the presence of the other whey protein fractions on the structure and barrier properties of the WPI films was analyzed. The effect of temperature on the OP of these films was also examined.

MATERIALS AND METHODS

Film Materials. WPI (>95% db protein) was obtained from Davisco Foods International (Le Sueur, MN). Glycerol (Gly) was obtained from Fisher Scientific, Inc. (Fair lawn, NJ). β -Lg (>95% purity) was separated from WPI at pH 7 following the procedure developed by Maté and Krochta (1994).

Film Formation. The method developed by McHugh and Krochta (1994) to make WPI edible films was used. Briefly, 10% (w/w) WPI of β -Lg aqueous solution was prepared and degassed under vacuum. The solution was maintained at 90 °C for 30 min in a water bath. After this, it was cooled to room temperature. Gly was then added to the solution as a plasticizer to avoid cracking of the dry films. The amount of Gly added was relative to the amount of protein (WPI or β -Lg) originally added to the solution. In WPI 60%/Gly 40% solution, there was a 60/40 ratio of WPI and Gly added. It was observed that when <15% Gly was added, the films obtained were too

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Table 1. Effect of Glycerol Amount on Water Vapor Permeability of WPI and β -Lg Films

film composition ^a	thickness ^b (μ m)	RH film underside ^c (%)	WVTR ^c (g/h m ²)	WVP ^{c,d} (g mm/kPa h m ²)
WPI 85%/Gly 15%	135 \pm 3.4	84 \pm 0.9	48.08 \pm 2.44	2.37 \pm 0.17 ^a
β -Lg 85%/Gly 15%	135 \pm 2.4	84 \pm 0.5	47.01 \pm 1.44	2.33 \pm 0.16 ^a
WPI 70%/Gly 30%	127 \pm 1.7	76 \pm 0.7	73.80 \pm 1.63	3.84 \pm 0.07 ^b
β -Lg 70%/Gly 30%	129 \pm 1.6	75 \pm 1.0	70.77 \pm 3.42	3.90 \pm 0.29 ^b
WPI 60%/Gly 40%	130 \pm 1.9	68 \pm 0.6	87.00 \pm 1.50	5.51 \pm 0.08 ^c
β -Lg 60%/Gly 40%	138 \pm 1.7	68 \pm 1.3	86.23 \pm 3.08	5.78 \pm 0.38 ^c

^a Percentages expressed in dry basis. ^b Mean values \pm standard error. ^c Mean values \pm standard deviation. ^d WVP with different letters are different at the 1% significance level.

brittle to be handled. To study the effect of Gly, three levels were used: 15%, 30%, and 40%. Solutions were degassed again before film casting.

Films were cast by pipetting WPI/Gly solution onto HDPE plates (14.7 cm i.d.) resting on a leveled granite surface. An amount of solution containing 2.6 g of total solids was pipetted per plate to minimize variations in the thickness of the films. Ten plates were cast per formula. The solutions were spread evenly with glass rods and allowed to dry overnight at room temperature (ca. 20 °C) and RH (ca. 50%). Dried films were peeled intact from the casting surface.

Film Thickness. Thickness of the films was measured by a caliper micrometer (No. 7326, Mitutoyo Manufacturing Co. Ltd., Japan) at six random positions following either WVP or OP tests. WVP and OP were calculated using the average thickness for each film replicate.

Water Vapor Permeability Test. The ASTM E96-80 method (ASTM, 1989) modified to estimate the RH at the film underside (McHugh et al., 1993) was used to measure WVP. Cabinets (Fisher Scientific) containing fans (Refrigeration Supply House, Sacramento, CA) were used to test WVP. The cabinets were placed in a controlled-temperature room held at 25 °C, and the cabinet fan speeds were set to achieve air velocities of 152 m/min to ensure uniform relative humidity inside the cabinets (McHugh et al., 1993). Prior to each experiment, cabinets were equilibrated to 0% RH using calcium sulfate desiccant (Fisher Scientific) during 24 h.

Circular cups used for the WVP tests were made of poly(methyl methacrylate) (Plexiglas). The external cup dimensions were 8.2 cm diameter and 1.25 cm height. The area of the cup mouth was 20.3 cm², and the cup well depth was 1.1 cm. A film was sealed to the cup mouth with a 8.2 cm diameter and 0.6 cm high Plexiglas ring with a 20.3 cm² opening using four screws, symmetrically located around the ring. Silicone sealant (High Vacuum Grease, Dow Corning, Midland, MI) was used between the cup and the film.

To expose films to high RH, 6 mL of deionized water was placed in the bottom of each test cup. After assembly, test cups with films were inserted into the pre-equilibrated 0% RH cabinets. McHugh et al. (1993) showed that for this kind of film, water transmission through the films achieved steady state in <2 h. Water loss through the films was measured by weight loss. Weights of test cups were recorded for 24 h over 6 periods of time, not less than 2 h apart. At least five replicates of each film were evaluated.

RHs at the film undersides and WVPs were calculated using the method developed by McHugh et al. (1993). The RH at the film underside was calculated using these three equations:

$$\text{WVTR} = \text{slope/film area} \quad (1)$$

$$\frac{\text{WVTR}}{\text{MW}} = \frac{PD \ln[(P - p_2)/(P - p_1)]}{RT\Delta z} \quad (2)$$

$$\text{RH}_{\text{underside}} = (p_2/p_1) \times 100 \quad (3)$$

In eqs 1–3, WVTR = water vapor transmission rate (g/h m²); slope = slope of weight loss vs time (g/h); film area = cup test mouth area (20.3 \times 10⁻⁴ m²); MW = molar weight of water (18 g/gmol); P = total pressure (1 atm); T = absolute temperature (298 K); D = diffusivity of water vapor through air at

298 K (1.02 \times 10⁻¹ m²/s); p_1 = saturation pressure of water vapor at 298 K (3.13 \times 10⁻² m²/s); p_2 = water vapor partial pressure at film underside (atm); R = gas constant (82.1 \times 10⁻⁶ m³atm/gmol K); Δz = mean stagnant air gap height ($Z_{\text{original}} + Z_{\text{final}}$)/2 (m).

Once p_2 was known, WVP was calculated using the following equation:

$$\text{WVP} = [\text{WVTR}/(p_2 - p_3)]L \quad (4)$$

p_3 = water vapor partial pressure at film overside (0% RH) (0 atm) and L = average film thickness (mm).

Oxygen Permeability Test. An Ox-Tran 2/20 modular system (Modern Control, Inc., Minneapolis, MN) was used to measure oxygen transmission rates through the films. Oxygen transmission rates were determined at various temperatures at a controlled RH. The RH selected was 40% to avoid cracking at very low RH and to decrease the masking effect of water at high RH. Aluminum masks with 5 cm² openings were used to avoid film cracking around the test cell seals. Masked films were placed into the test cell and exposed to pure nitrogen flow on one side and pure oxygen flow on the other. The humidities of both gases were adjusted by the Ox-Tran 2/20 to 40% RH before they contacted the films. The Ox-Tran 2/20 was programmed to have a 8 h waiting period to allow the film to achieve equilibrium with the RH. The system measured the amount of oxygen transferred through the film to the nitrogen flow. OP was calculated by dividing the oxygen transmission rate by the difference in oxygen partial pressure between the sides of the film (1 atm) and multiplying by the average film thickness. Three replicates of each film were evaluated at four different temperatures (15, 23, 30, and 37 °C).

Arrhenius Model. The temperature dependence of the OP was described by an Arrhenius model (Rogers, 1985; Gennadios et al., 1993; Torres, 1994)

$$\text{OP} = \text{OP}_0 \exp(-E_a/RT) \quad (5)$$

where E_a is the activation energy, R is the universal gas constant (1.987 cal/mol K), T is the absolute temperature (K), and OP_0 is an Arrhenius constant (cm³ μ m/kPa m² day). Activation energies and Arrhenius constants were determined by Arrhenius plots of $\ln(\text{OP})$ vs $1/T$ for each type of film.

Statistical Analysis. Statview 4.0 (Abacus Concepts, Berkeley, CA) was used for all statistical analyses. Adequacy of the Arrhenius model was determined by R^2 value.

RESULTS AND DISCUSSION

Water Vapor Permeability. A two-factor (protein type and amount of Gly) analysis of variance was performed with the experimental data (Table 1). WVPs of WPI films were not statistically different ($p < 0.01$) from WVPs of β -Lg films. The presence of WPI fractions other than β -Lg (α -La, BSA, Igs, PP) in the protein matrix of WPI films has the same effect on WVP as the presence of β -Lg.

The amount of Gly affected WVP significantly ($p < 0.01$), with a nearly linear relation between the amount of Gly in the film formulation and WVP (Figure 1). This is not a direct comparison, because the RH at the

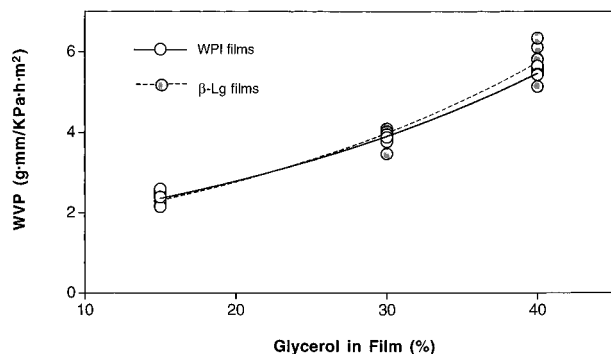


Figure 1. Effect of glycerol content on the WVP of WPI or β -Lg films.

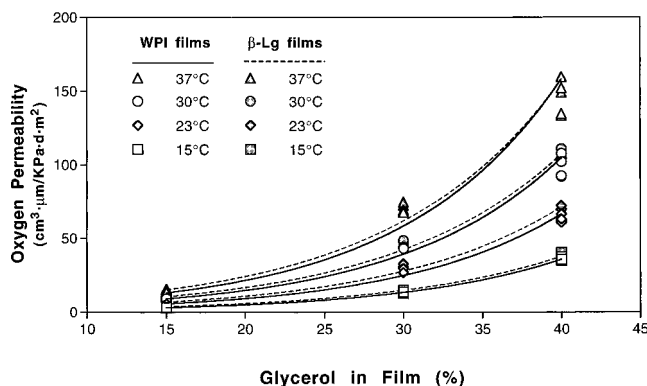


Figure 2. Effect of glycerol content on the OP of WPI or β -Lg edible films.

underside is different for different amounts of plasticizer (Table 1). The higher the RH at the underside, the higher the moisture content of the films (Yun, 1995). WVP of hydrophilic films (e.g., those based on WPI or β -Lg) increases with increased moisture content because of the plasticizing effect of the water (McHugh et al., 1993). In the present study, the films with the highest Gly content were tested at the lowest RH at the underside ($\approx 68\%$); therefore, they had lower moisture content than if they had been tested at the RH of films with the lowest Gly content ($\approx 84\%$). As a consequence, if all films were tested at the same underside RH, an exponential-type relationship between WVP and Gly content would be expected for both WPI and β -Lg films. Note that the presence of Gly, although necessary to avoid cracking, could have masked differences in WVP between WPI and β -Lg films.

Our results for WPI films were similar to those obtained by McHugh and Krochta (1994). This indicates good repeatability, even though WPI came from different batches.

Oxygen Permeability. The amount of Gly affected the OP of both WPI and β -Lg films in an exponential fashion (Figure 2). Temperature also had an exponential effect on the OP of both WPI and β -Lg films at the three studied Gly levels (Figure 3). OPs of WPI films appeared lower than those of β -Lg films with the same Gly content. The lower the amount of Gly, the higher the relative difference between WPI and β -Lg films (Figure 3). The influence of large amounts of plasticizer on the OP is probably sufficiently important that it masks any difference between WPI and β -Lg films. However, lower amounts of Gly would have made films too brittle to handle.

To evaluate the effect of protein type on OP, the E_a and OP_0 in the Arrhenius model were determined by fitting OPs of individual films. A t -test was performed

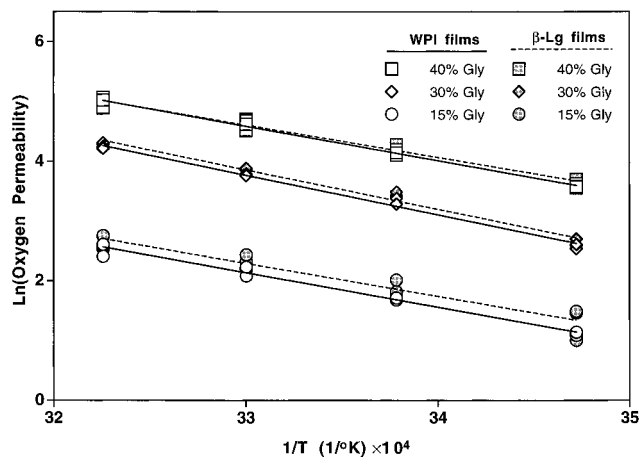


Figure 3. Arrhenius plots for the OP of WPI and β -Lg films with different amounts of glycerol.

with the resulting E_a and OP_0 of WPI and β -Lg films for each Gly content. E_a and OP_0 of WPI and β -Lg films with same Gly content were not significantly different ($p < 0.01$) at all studied Gly contents. As with WVP, the presence of β -Lg in the protein structure of WPI has the same effect on the OP as the presence of the other WPI fractions, with the amount of Gly used.

Intermolecular disulfide bonds formed during heat treatment are partly responsible for film structure (McHugh et al., 1994). β -Lg contains two disulfide bonds and one free thiol group which can form intermolecular bonds during the heat treatment. The other protein fractions contain different numbers of disulfide bonds and thiol groups (Dybing and Smith, 1991), which can also contribute to different degrees of interconnectedness of the WPI film structure. Since there is no difference in WVP and OP between β -Lg and WPI films, differences in molecular structure between β -Lg and WPI films appear to have no effect on transport of water vapor or oxygen through these materials.

There was a good fit of the experimental OPs with the Arrhenius model, shown by R^2 ranging from 0.925 to 0.990 (Table 2). The Arrhenius model applied to OP has been confirmed for other edible films (Gennadios et al., 1993) as well as for synthetic films (Pauly, 1989). E_a values for moisture and gas permeabilities are generally expected to be in the 0–15 kcal/mol range (Torres, 1994). E_a values of both WPI and β -Lg films are close to the upper limit of that range (Table 2), which means that whey protein films are more sensitive to temperature than other films with lower E_a . These E_a values are similar to the ones found for other protein-based edible films (Gennadios et al., 1993).

Increased amount of Gly reduces polymer interchain attraction forces. As a consequence, the energy necessary to transfer oxygen through the film is decreased. This is generally reflected in a reduced E_a (Torres, 1994). E_a decreased as Gly increased from 30% to 40%. However, the E_a for 15% Gly-containing films was also lower than that of 30% Gly content (Table 2). Permeability is a combined process of sorption, diffusion, and desorption. Temperature may affect the sorption/desorption and diffusion processes in a different fashion. If diffusion activation energies had been obtained instead of permeability activation energies, the effect of Gly probably would have been more consistent with the theory (i.e., a higher amount of Gly would have given a lower diffusion activation energy).

Arrhenius plots can also be used to locate glass transition temperatures (T_g). T_g identifies transition

Table 2. Parameters and R^2 of Fitted Arrhenius Models for WPI and β -Lg Film Oxygen Permeability

film composition ^a	thickness ^b (μm)	Arrhenius		R^2
		constant ($\text{cm}^3 \mu\text{m}/\text{kPa}$ d m^2) $\times 10^9$	activation energy (kcal/mol)	
WPI 85%/Gly 15%	115 \pm 1.9	1.6	11.48	0.985
β -Lg 85%/Gly 15%	114 \pm 5.8	0.9	11.01	0.925
WPI 70%/Gly 30%	134 \pm 1.3	149	13.21	0.990
β -Lg 70%/Gly 30%	138 \pm 2.6	100	13.15	0.982
WPI 60%/Gly 40%	111 \pm 5.8	20	11.46	0.989
β -Lg 60%/Gly 40%	111 \pm 6.7	6.2	10.81	0.979

^a Percentages expressed in dry basis. ^b Mean values \pm standard error.

from glass behavior to rubbery and more permeable behavior. At T_g , there is a break in the relation between OP and T , and, as a consequence, a change in the slope of the Arrhenius model (Karel, 1975). Since there was no change in the slope of the Arrhenius plots, we can conclude that T_g is not in the range 15–37 °C for any of the studied films.

Conclusions. Despite the different molecular structures of films based on β -Lg and WPI, oxygen and water vapor permeabilities between both films are not statistically different. It can be concluded that β -Lg and the other WPI fractions likely contribute equally to film barrier properties. The amount of glycerol was a more critical factor than the type of protein. Increased amounts of glycerol in the film formulation resulted in exponential increases of both WVP and OP. The presence of glycerol, although necessary to analyze intact films, could have masked some differences between WPI and β -Lg films.

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