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Sorbitol- vs Glycerol-Plasticized Whey Protein Edible Films: Integrated Oxygen Permeability and Tensile Property Evaluation

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An integrated approach for evaluation of the oxygen permeability and tensile properties of plasticized whey protein-based edible films was developed. Relative humidity had an exponential effect on the oxygen permeability of whey protein films. The increase in oxygen permeability caused by increasing concentrations of glycerol was greater than that of sorbitol. Increasing concentrations of both glycerol and sorbitol resulted in significantly decreased tensile strength and increased elongation. Sorbitol was more effective than glycerol as a plasticizer in that films of equal tensile strength, elongation, and elastic modulus had lower oxygen permeabilities when plasticized using sorbitol. The oxygen permeability and tensile properties of whey protein-based films compared favorably with those of other protein and synthetic film materials.

INTRODUCTION

The potential of edible films to control mass transfer to improve food quality and extend food shelf life has recently been reviewed by Guilbert (1986), Kester and Fennema (1986), and Krochta (1992). In the past, most edible film research has focused on the water vapor permeability properties of film materials. Oxygen permeability properties have been less extensively investigated. Food spoilage from degradative oxidation and respiration reactions can potentially be controlled through the use of edible films. Protein and polysaccharide films are expected to be excellent oxygen barriers, due to their tightly packed, ordered hydrogen-bonded network structure and low solubility. Lipids, on the other hand, offer limited oxygen barrier properties, due to the presence of microscopic pores (Banker, 1966) and elevated solubility and diffusivity.

The potential of whey proteins as mass-transfer barriers has recently generated interest due to their favorable nutritional characteristics, functional properties, and

industrial surplus. Mahmoud and Savello (1992, 1993) formed whey protein films using transglutaminase as a cross-linking agent and investigated the mechanical properties, water vapor transferability, solubility, and hydrolyzability of resultant films. The expense of transglutaminase limits its potential use in food systems. McHugh et al. (1994) developed methods for production of the first whey protein edible films without the use of transglutaminase. Thiol-disulfide exchange and thiol oxidation reactions were induced through heat treatment, resulting in the formation of intermolecular disulfide bonds necessary to form intact films. The water vapor permeability properties of plasticized whey protein films were reported (McHugh et al., 1994). Films formed from heat denaturation of whey were brittle, necessitating the use of plasticizers to increase film flexibility.

A plasticizer is defined as "a substantially nonvolatile, high boiling, nonseparating substance, which when added to another material changes the physical and/or mechanical properties of that material" (Banker, 1966). Polyols, such as sorbitol and glycerol, plasticize effectively due to their ability to reduce internal hydrogen bonding while increasing intermolecular spacing (Lieberman and Gilbert, 1973). Plasticizers are theorized to decrease the intermolecular forces along polymer chains, imparting increased film

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flexibility while decreasing the barrier properties of films (Banker, 1966). Therefore, an integrated approach, evaluating permeability and mechanical property data in concert, must be performed for complete characterization of plasticizer effectiveness. In the past, such an approach has not been implemented.

The objective of this study was to utilize an integrated approach to evaluate the oxygen permeability and tensile properties of plasticized whey protein-based edible films to compare the effectiveness of glycerol and sorbitol as plasticizers. The effect of relative humidity on the oxygen permeability was also characterized.

MATERIALS AND METHODS

Materials. BiPRO whey protein isolate used to make films was provided by Le Sueur Isolates (Le Sueur, MN). Sorbitol and glycerol plasticizers were obtained from Fisher Scientific, Inc. (Fair Lawn, NJ). Magnesium nitrate saturated salt solution was used to equilibrate films at $50 \pm 5\%$ relative humidity prior to tensile property testing.

Film Formation Method. Aqueous solutions of 10% (w/w) whey protein isolate (WPI) were prepared and heated at 90°C for 30 min in a water bath (Lauda MS circulator with Lauda MA5 bath, Fisher Scientific). Solutions were then cooled at room temperature, and a vacuum was applied to remove dissolved air. Weights of sorbitol (S) or glycerol (G) relative to the weight of WPI originally dissolved were then added as plasticizers for films. For example, equal weights of WPI and S would yield 50% WPI/50% S films.

Films were cast by pipetting solutions onto smooth, rimmed, 14.7 cm i.d. poly(methyl methacrylate) (Plexiglas) plates sitting on a leveled granite surface. All whey protein solutions were applied at 2.625 g of total solids per plate to minimize thickness variations between treatments. Solutions were spread evenly with a bent glass rod and allowed to dry for approximately 18 h at 23°C and 40% relative humidity. Dried films could be peeled intact from the casting surface.

Film Thickness Measurement. Film thicknesses were measured with a micrometer (L. S. Starrett Co., series 436, catalog no. T436RL-1, Athol, MA) to the nearest 0.0001 in. ($2.54\ \mu\text{m}$) at five random positions around the film, and average values were used in calculations. Average film thickness was 0.0044 in. ($110\ \mu\text{m}$). Thickness measurements varied up to 5%. Film thickness was measured after oxygen permeability tests and after equilibration at 50% relative humidity for tensile property tests.

Oxygen Permeability Determination. An Ox-Tran 2/20 ML modular system (Modern Controls, Inc., Minneapolis, MN) was utilized to measure oxygen transmission rates through plasticized whey protein films according to method D3985 of the American Society of Testing and Materials (ASTM, 1988). Oxygen transmission rates were determined at 23°C under variable relative humidity conditions. Films were placed into the oxygen test cell and exposed to pure nitrogen on one side of the film and pure oxygen on the other. Both gas streams were humidified to the desired conditions. The area of film tested was controlled with adhesive aluminum masks having 5-cm² openings. Films cracked around the seals when aluminum masks were not utilized.

Oxygen permeability was calculated by dividing the oxygen transmission rate by oxygen pressure and multiplying by the film thickness.

Tensile Property Investigations. Films were preconditioned at approximately 90% relative humidity for 2 h at $23 \pm 2^\circ\text{C}$. The films were then cut into 15 mm wide strips using a razor blade. The ends of each of these strips were mounted between cardboard grips (26 mm by 68 mm) using double-stick tape. The cardboard padded the films against cracking when clamped into the metal grips of the Instron. The final film area exposed was 15 mm by 90 mm. These mounted films were then equilibrated overnight at $50 \pm 5\%$ relative humidity and $23 \pm 2^\circ\text{C}$ in an environmental chamber (Fisher Scientific, catalog no. 08-647-24) containing magnesium nitrate saturated salt solution. Ten replicates of each film type were tested.

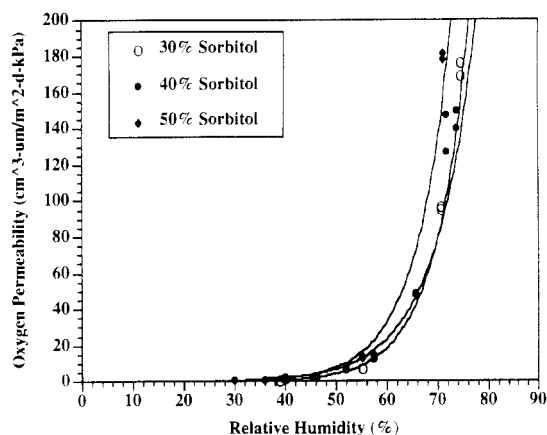


Figure 1. Effect of sorbitol concentration on the relationship between relative humidity and oxygen permeability in plasticized whey protein edible films at 23°C .

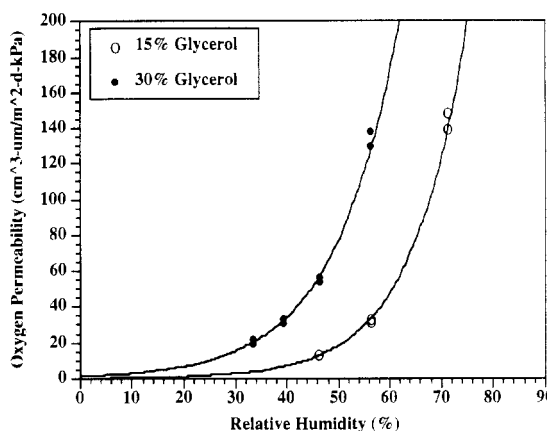


Figure 2. Effect of glycerol concentration on the relationship between relative humidity and oxygen permeability in plasticized whey protein edible films at 23°C .

An Instron universal testing instrument Model 1122 (Instron Engineering Corp., Canton, MA) was used to measure the tensile properties of edible films according to ASTM standard method D882 (ASTM, 1991). Test method A, the static weighing, constant-rate-of-grip separation test, was employed. The room in which the Instron is located was equilibrated to $50 \pm 5\%$ relative humidity and $23 \pm 2^\circ\text{C}$. The mounted films were clamped into the metal grips of the Instron. Initial grip separation and crosshead speed were set to 75 mm and 100 mm/min, respectively. Tensile strength at break, percent elongation at break, and elastic modulus were calculated as described by ASTM D882 (ASTM, 1991).

Statistical Analyses. StatView 4.0 was employed for all statistical analyses (Abacus Concepts, Berkeley, CA). Analysis of variance and Fisher PLSD multiple-comparison tests were utilized.

RESULTS AND DISCUSSION

Oxygen Permeability Properties. An exponential function was fit to the oxygen permeability data of plasticized whey protein edible films at increasing relative humidity conditions (Figures 1 and 2). The plasticizing and/or swelling effect of water on hydrophilic (polar) polymers, such as whey proteins, resulted in increased permeability values (Ashley, 1985). Relationships such as those shown in Figures 1 and 2 are critical for modeling the behavior of edible films for application to food products having different water activities and/or stored under different relative humidity conditions.

The exponential effect of relative humidity on oxygen permeability had been previously observed for other edible

Table 1. Oxygen Permeability (O₂P) and Tensile Properties of Whey Protein-Based Edible Films Compared to Other Protein-Based Edible Films and Synthetic Films

	film ^a	test conditions for O ₂ P	O ₂ P ^b (cm ³ ·μm/m ² ·d·kPa)	tensile strength (MPa)	elongation (%)
present study	WPI:G (5.7:1)	23 °C, 50% RH ^c	18.5	29.1	4.1
present study	WPI:G (2.3:1)	23 °C, 50% RH	76.1	13.9	30.8
present study	WPI:S (2.3:1)	23 °C, 50% RH	4.3	14.0	1.6
present study	WPI:S (1:1)	23 °C, 50% RH	8.3	14.7	8.7
present study	WPI:BW:S (3.5:1.8:1)	23 °C, 50% RH	11.6		
present study	WPI:S (3.5:1)	23 °C, 40% RH	0.7		
present study	WPI:S (3.5:1)	23 °C, 70% RH	43.3		
Mahmoud and Savello (1993)	Blg:G (2.6:1), Tgase			141.2	
Gennadios et al. (1991)	WG:G (2.5:1)	23 °C, 0% RH	3.8	2.6	238
Gennadios et al. (1993)	CZ:G (5:1)	23 °C, 0% RH	7.7		
Lieberman and Gilbert (1973)	collagen	23 °C, 63% RH	2.3		
Lieberman and Gilbert (1973)	collagen	23 °C, 93% RH	89.0		
Salame (1986)	LDPE	23 °C, 50% RH	1870	13	500
Salame (1986)	HDPE	23 °C, 50% RH	427	26	300
Taylor (1986)	cellophane	23 °C, 95% RH	252		
Salame (1986)	EVOH (70% VOH)	23 °C, 0% RH	0.1		
Salame (1986)	EVOH (70% VOH)	23 °C, 95% RH	12		
National Bureau of Standards	polyester 1470	23 °C, 0% RH	17.3		

^a Abbreviations: WPI, whey protein isolate; G, glycerol; S, sorbitol; BW, beeswax; Blg, β-lactoglobulin; Tgase, transglutaminase cross-linked; WG, wheat gluten; CZ, corn zein; LPDE, low-density polyethylene; HDPE, high-density polyethylene; EVOH, ethylene vinyl alcohol; VOH vinyl alcohol. ^b Calculated at atmospheric pressure and 23 °C. ^c RH, relative humidity.

film types. Rico-Pena and Torres (1990) examined the effect of relative humidity on the oxygen transmission rate through methylcellulose-palmitic acid edible films. No significant increase in oxygen transmission rate was observed between 0 and 57% relative humidity. However, above 57% relative humidity an exponential increase in oxygen transmission rate resulted. This increase correlated with the moisture isotherm for methylcellulose-palmitic acid films. Hagenmaier and Shaw (1991) found an exponential increase in oxygen permeability of shellac coatings with increasing relative humidity. Relative humidity increases from 63 to 93% resulted in significantly increased oxygen permeabilities in collagen films (Table 1) (Lieberman and Gilbert, 1973).

The effect of plasticizer concentration on oxygen permeability was also examined (Figures 1 and 2). Higher sorbitol than glycerol concentrations were required to compare the permeability properties of films exhibiting similar tensile properties. The smaller size of glycerol enables it to influence film mechanical properties more readily than sorbitol. Therefore, glycerol concentrations of 15 and 30% and sorbitol concentrations of 30, 40, and 50% were utilized. Sorbitol films exhibited lower oxygen permeability values than glycerol films at all relative humidities, even though sorbitol concentrations were higher (Figures 1 and 2). Table 1 compares the oxygen permeabilities of plasticized whey protein films at 50% relative humidity and 23 °C. The 30% glycerol films showed significantly higher oxygen permeabilities than 30% sorbitol films.

Glycerol concentration exhibited an exponential effect on the carbon dioxide permeability properties of collagen films (Lieberman and Gilbert, 1973). Glycerol was hypothesized to compete with water for active sites on the polymer, thus promoting water clustering and increased free volume in the polymers at low moisture levels (Lieberman and Gilbert, 1973). The effect of glycerol concentration on diffusivity was greater than that on solubility, supporting the hypothesis that free volume changes resulted in the increased permeability values (Lieberman and Gilbert, 1973).

Alternatively, glass transition theory can be utilized to explain plasticizer effects on permeability properties. Glass transitions occur when a polymer structure changes from a brittle glass to a highly viscous or rubbery solid (Slade

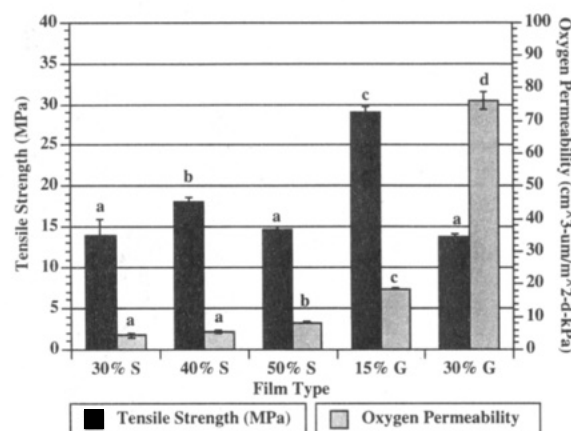


Figure 3. Effect of plasticizer type and concentration on the tensile strength (MPa) and oxygen permeability (cm³·μm/m²·d·kPa) of whey protein-based edible films at 50% relative humidity. Statistical analyses of tensile strength and oxygen permeability for the different film types were performed separately. Standard error bars are shown. a–d represent significantly different values at *p* < 0.05 using the Fisher PLSD multiple-comparison method.

and Levine, 1991). Glass transition temperatures of proteins are known to be lowered by the plasticization of water (Gontard et al., 1993). Water increases the free volume of the polymer, thereby increasing polymer mobility and permeability. Polyol plasticizers are hypothesized to act in the same manner. The temperature dependence of oxygen permeability and tensile properties should be examined to better relate glass transition theory to edible film properties.

Tensile Properties. Tensile strength, elongation, and elastic modulus can be used to describe how the mechanical properties of film materials relate to their chemical structures (Ninnemann, 1968). Tensile strength is the maximum tensile stress that a film can sustain. Elongation is the maximum change in length of the test specimen before breaking. Elastic modulus is the fundamental measure of film stiffness (Hay, 1968). The tensile data in Figures 3–5 are presented along with oxygen permeability data to fully compare the effectiveness of sorbitol vs glycerol in plasticized whey protein edible films under 50% relative humidity conditions.

At equal concentrations, 30% sorbitol-plasticized whey

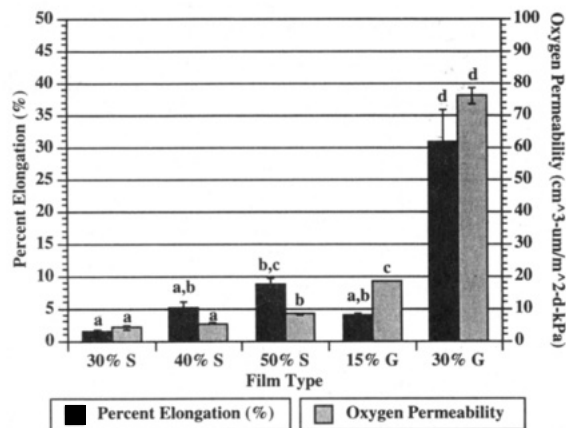


Figure 4. Effect of plasticizer type and concentration on the percent elongation (%) and oxygen permeability ($\text{cm}^3 \cdot \mu\text{m} / \text{m}^2 \cdot \text{d} \cdot \text{kPa}$) of whey protein-based edible films at 50% relative humidity. Statistical analyses of percent elongation and oxygen permeability for the different film types were performed separately. Standard error bars are shown. a-d represent significantly different values at $p < 0.05$ using the Fisher PLSD multiple-comparison method.

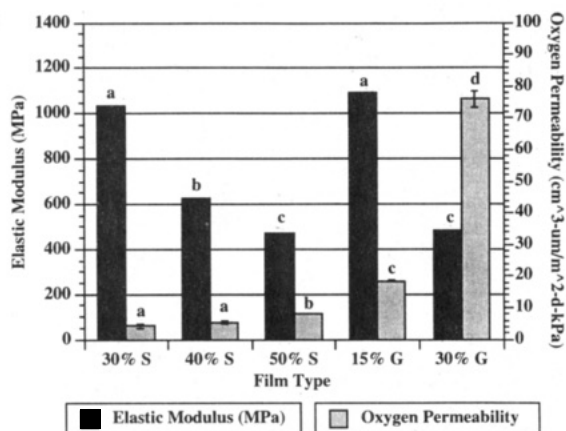


Figure 5. Effect of plasticizer type and concentration on the elastic modulus (MPa) and oxygen permeability ($\text{cm}^3 \cdot \mu\text{m} / \text{m}^2 \cdot \text{d} \cdot \text{kPa}$) of whey protein-based edible films at 50% relative humidity. Statistical analyses of elastic modulus and oxygen permeability for the different film types were performed separately. Standard error bars are shown. a-d represent significantly different values at $p < 0.05$ using the Fisher PLSD multiple-comparison method.

protein films had tensile strengths equivalent to those of glycerol-plasticized films (Figure 3). As plasticizer concentration increased, tensile strength decreased significantly for glycerol (Figure 3).

The percent elongation increased significantly as plasticizer concentration increased in both sorbitol- and glycerol-plasticized whey protein films (Figure 4). At 30% plasticizer concentrations, glycerol films exhibited higher elongation values than sorbitol plasticized films (Figure 4).

The elastic modulus of sorbitol-plasticized films was significantly greater than that of glycerol films at 30% concentration (Figure 5). Furthermore, as plasticizer concentration increased, film elastic modulus decreased and the film flexibility increased with both sorbitol and glycerol (Figure 5).

Integrated Evaluation of Tensile and Permeability Properties. The effects of plasticizer type and concentration on the tensile properties of films were discussed in the preceding paragraphs. Alone, these values possess limited value. However, by integrating them with permeability data, the potential of these films can be more

effectively evaluated. This approach has not been explored in the past by edible film researchers.

The oxygen permeabilities of sorbitol- vs glycerol-plasticized films of equal tensile strength, elongation, and elastic modulus values can be compared in Figures 3-5. The 30% glycerol films exhibited statistically equivalent tensile strengths to both 30 and 50% sorbitol films, yet glycerol plasticized films exhibited much greater oxygen permeabilities. The 15% glycerol films exhibited statistically equivalent elongation values to all sorbitol films, yet glycerol-plasticized films once again exhibited greater oxygen permeabilities. The elastic moduli of 15 and 30% glycerol films were equivalent to those of 30 and 50% sorbitol films, respectively, but the oxygen permeabilities of the glycerol films were greater than those of sorbitol films in each case. On the basis of tensile strength, percent elongation, and elastic modulus, sorbitol was a more effective plasticizer than glycerol in whey protein film systems with regard to oxygen barrier properties.

For application of films to food systems it is important to develop films possessing favorable mechanical and permeability characteristics. Therefore, combined analyses are crucial for predicting film behaviors and defining structure/function relationships.

In this study, percent elongation for different film types correlated linearly with oxygen permeability values ($R^2 = 0.93$); however, not enough film types were examined to formulate widespread conclusions. Both free volume theory and molecular theory relate film mechanical and permeability properties. In the future, relationships between film mechanical and permeability properties will be developed to enable accurate prediction of and further the understanding of edible film behaviors.

Comparison of Protein and Synthetic Films Properties. Table 1 compares the oxygen permeability and tensile properties of whey protein films with those of other protein-based edible films and synthetic films. The addition of beeswax to whey protein films resulted in significantly increased oxygen permeability. Wheat gluten/glycerol films had lower tensile strengths and greater elongation values than whey/glycerol films. All protein-based films were excellent oxygen barriers. The oxygen permeabilities of whey and other protein films were lower than those high of density polyethylene and compared favorably to those of ethylene vinyl alcohol films. Therefore, the use of protein-based films for the control of oxidation and respiration in food systems is an extremely promising area of research.

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