Oxygen Permeability and Mechanical Properties of Films from Hydrolyzed Whey Protein

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The effects of whey protein hydrolysis on film oxygen permeability (OP) and mechanical properties at several glycerol—plasticizer levels were studied. Both 5.5% and 10% degree of hydrolysis (DH) whey protein isolate (WPI) had significant effect ($p \leq 0.05$) on film tensile properties compared to unhydrolyzed WPI. Hydrolyzed WPI required less glycerol to achieve the same mechanical properties compared to those of unhydrolyzed WPI. Little or no significant difference ($p > 0.05$) occurred for film OP between unhydrolyzed WPI, 5.5% DH WPI, and 10% DH WPI films at the same glycerol content. Hydrolyzed WPI films of mechanical properties similar to those of WPI films had better oxygen barrier. Therefore, use of hydrolyzed WPI allowed achievement of desired film flexibility with less glycerol and with smaller increase in OP.

Keywords: *Hydrolyzed whey protein; oxygen permeability; mechanical properties*

INTRODUCTION

The main advantages of using edible films and coatings are extending food shelf life, improving food quality, adding value to the edible film-forming polymer, and reducing synthetic packaging materials (Donhowe and Fennema, 1993; Krochta, 1997a). In general, edible films and coatings provide the potential to control transport of moisture, oxygen, aroma, oil, and flavor compounds in food systems, depending on the nature of the edible film-forming materials (Donhowe and Fennema, 1993; Krochta, 1997b; Krochta and De Mulder-Johnston, 1997c). However, data have been lacking on film properties of specific materials, and such data are absolutely important for food applications to occur. Specifically, fundamental studies are still needed to investigate film composition—structure—function relations as a critical step toward food application.

Whey protein has received much attention because it has been shown to make transparent films and coatings with excellent oxygen, aroma, and oil barrier properties (McHugh et al., 1994; Mate and Krochta, 1996a; Mate and Krochta, 1996b; Miller and Krochta, 1997; De Mulder-Johnston, 1999). However, proteins by themselves, including whey protein, form brittle films, which are useless in application. The usual approach is to add a plasticizer, which is a low-molecular-weight nonvolatile substance, into the film to reduce protein chain-to-chain interaction. The result is increase in the mobility of polymer chains and more flexible films (Banker, 1966). However, plasticizers also increase the film permeability (Gontard et al., 1993), especially plasticized hydrophilic films. Increasing permeability is undesirable for food quality, so there is a need to minimize the use of plasticizers. Another potential approach to increasing film flexibility is reducing polymer molecular weight, thus reducing intermolecular forces along polymer chains and increasing polymer chain end groups and polymer free volume (Sears and Darby, 1982). This approach may permit a decrease in the required amount of added plasticizer in films; consequently, it may minimize permeability of films while producing needed film flexibility. Previous work showed that hydrolyzed whey protein isolate (WPI) required less plasticizer than unhydrolyzed WPI to form intact films with desirable mechanical properties (Sothornvit and Krochta, 2000).

The low oxygen permeability (OP) of whey protein films is a very useful property, but the OP is increased with the increased levels of plasticizer required for some applications. Simultaneous investigation of film OP along with mechanical properties as a function of plasticizer content would show whether hydrolyzed WPI films with desirable mechanical properties could be achieved with less plasticizer and thus lower OP than unhydrolyzed WPI films. In addition, hydrolyzed WPI has greater solubility and emulsifying ability and improved digestibility (Nielsen, 1997; Singh and Dalgleish, 1998). Furthermore, use of hydrolyzed WP reduces allergic response due to the destruction of globular protein structure during hydrolysis (Nielsen, 1997).

The objective of this study was to determine the effect of whey protein degree of hydrolysis DH on film OP and mechanical properties. Little or no change in film OP while giving desirable film mechanical properties would be an indication of a very useful effect of whey protein molecular weight (M_w) reduction.

MATERIALS & METHODS

Materials. BiPro WPI (97.7% protein dry basis (db)) and 5.5% degree of hydrolysis (DH) WPI (96.3% protein db) supplied by Davisco Foods International (Le Sueur, MN) and 10% DH WPI (90.1% protein db and $M_w = 1100$ as declared by company) supplied by New Zealand Milk Products (Santa Rosa, CA) were used to make films. Both hydrolyzed products are produced enzymatically. Glycerol (gly) as a plasticizer was

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purchased from Fisher Scientific, Inc., Fair Lawn, NJ. A saturated salt solution of magnesium nitrate (Fisher Scientific Inc.) was used to equilibrate films to an environment at 50 ± 5% relative humidity (RH) prior to tensile testing.

Film Formation. Aqueous solutions (10% w/w) of either WPI, 5.5% DH WPI, or 10% DH WPI were heated at 90 °C for 30 min in a water bath (Lauda MS circulator with MA5 bath, Fisher Scientific) (McHugh and Krochta, 1994). Solutions were cooled to room temperature and degassed by applying vacuum to remove dissolved air, followed by adding 25, 30, 35, 45 or 55% (w/w) of gly plasticizer and then degassing again. A volume of solution containing 3 g of total solids was pipetted onto 14.7 cm internal diameter, rimmed, smooth high-density polyethylene (HDPE) casting plates to form films to determine film OP. A volume of solution containing 5 g of total solids was pipetted onto 30 × 30 cm^2 rimmed, smooth HDPE casting plates to form films to determine film mechanical properties. All casting plates were placed on a leveled surface at room temperature (23 °C and 35 ± 5% RH) until dried films could be released intact from plates. Three replications were used to determine each property.

Film Thickness. Film thickness was measured with a micrometer (No. 7326, Mitutoyo Manufacturing Co., Ltd., Japan) to ± 0.0001 in (0.00254 mm) around the film testing area at five random positions. An average of film thickness was used to determine OP and mechanical properties for each film replicate. The average thickness of films was 0.1344 ± 0.01 mm for mechanical property tests and 0.0130 ± 0.001 mm for OP tests.

Oxygen Permeability Measurement. OP of plasticized WPI films was measured at 23 °C and 50 ± 1% RH using an Ox-tran 2/20 ML modular system (Modern Controls, Inc., Minneapolis, MN) according to the American Society of Testing and Materials Standard Method D3985 (ASTM, 1995). A film was placed on a stainless steel mask with an open testing area of 5 cm^2. One side of the film was exposed to flowing nitrogen gas, and the other side was exposed to flowing oxygen gas at the above condition.

Mechanical Properties Measurement (Tensile Test). An Instron universal testing machine (model 1122, Instron Corp., Canton, MA) was used to determine tensile strength (TS or stress at maximum before break), elastic modulus (EM or Young's modulus), and percent elongation (%*E*) according to ASTM standard method D882 (ASTM, 1997). Maximum TS is the largest stress that a film is able to sustain. EM, which is the ratio of stress to strain in the elastic region, reflects the film stiffness (Steffe, 1996). The %*E* is the maximum percent change in length of film before breaking.

Preconditioning. Preconditioning at 50% RH with saturated salt solution was needed to provide ease of cutting films. Films, cast on square plates, were cut by using a striking die (The Right Image, Sacramento, CA). The film shape was a rectangular center, 15 mm wide by 100 mm long, flaring to 25 × 35 mm^2 grip areas on both ends. Film strips were equilibrated at 23 ± 2 °C and 50 ± 5% RH for at least 48 h in a controlled cabinet using saturated magnesium nitrate solution prior to a test.

Test Condition. An Instron universal testing machine with a 500 kg load cell was operated at 23 ± 2 °C and 50 ± 5% RH with self-alignment grips (model 2712-002,). Self-alignment grips consist of one fixed end and one movable end in such a way that they will move easily into a perfect alignment position when load is applied. The grips are lined with thin rubber faces to prevent film slippage and air-actuated to prevent film breakage at the neck. The initial gauge separation and crosshead speed were set to 115 mm and 50 mm/min, respectively.

Statistical Analyses. A completely randomized experimental design was used to study the following factors: (1) WPI type, (2) gly content, and (3) interaction between WPI type and gly content. SAS system software program, release 6.12 (SAS Institute Inc., 1996) was utilized to calculate analysis of variance (ANOVA) using the general linear models procedure PROC GLM, and a Duncan's multiple range test was used to determine any significant difference among experimental treatments at $p \leq 0.05$.

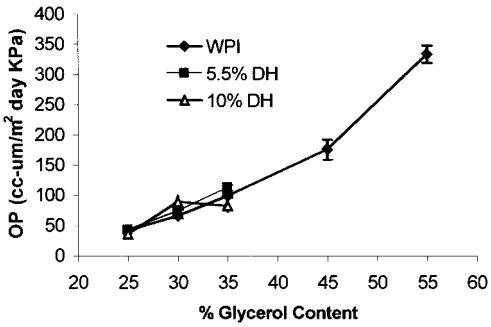

Figure 1. Effect of WPI DH on OP of edible films. Error bar shows standard deviation.

RESULTS & DISCUSSION

Hydrolyzed WPI materials were defined by the degree of hydrolysis in percent (% DH) which is the percentage of peptide bonds broken (Adler-Nissen, 1979). In our study, 5.5% DH WPI and 10% DH WPI were used. It was shown previously that, like unhydrolyzed WPI, hydrolyzed WPI without plasticizer could not form flexible stand-alone films (Sothornvit and Krochta, 2000). Nevertheless, hydrolyzed WPI required less plasticizer than unhydrolyzed WPI to achieve desirable film flexibility. Too much plasticizer made hydrolyzed WPI films sticky and difficult to peel off the casting plates.

OP. Oxygen initiates lipid oxidation, which decreases food quality and shortens food shelf life. Figure 1 shows that films had very little difference in OP at the same gly contents among the WPI types. OP of WPI and 10% DH WPI films was not significantly different ($p > 0.05$) at any gly content, except for small difference between WPI and 5.5% DH WPI at 35% gly. OP values of WPI films in this study were similar to the McHugh and Krochta results (1994) with 30% gly plasticized WPI films. No significant difference was found in film OP for all WPI types at 25% gly. These results show the same trend as WPI film water vapor permeability (WVP) in previous work (Sothornvit and Krochta, 2000). Thus, unlike the addition of plasticizer, WPI hydrolysis has little or no effect on film WVP or OP. Furthermore, these results confirm that for films with equal plasticizer amount, whey protein composition and structure have little or no effect on film permeability (Mate and Krochta, 1996a; Perez-Gago et al., 1999; Sothornvit and Krochta, 2000). Donhowe and Fennema (1993) concluded that factors affecting OP of plasticized methyl cellulose films were the physical state and the M_w of the plasticizer, the chemical interaction between plasticizer and oxygen, and the type of film structure. It can be concluded here that type of film structure (unhydrolyzed vs hydrolyzed WPI) did not affect film OP with the same plasticizer.

To clarify the effect of WP DH on film properties, additional films were studied at 45 and 55% gly with unhydrolyzed WPI for both OP (Figure 1) and mechanical properties (Figures 2−4). Adding 45 and 55% gly to the hydrolyzed WPI materials gave very weak films, which were extremely sticky.

Mechanical Properties. Mechanical properties are important for edible films and film coating, as they reflect the durability of films and the ability of the coating to enhance mechanical integrity of foods. Unlike

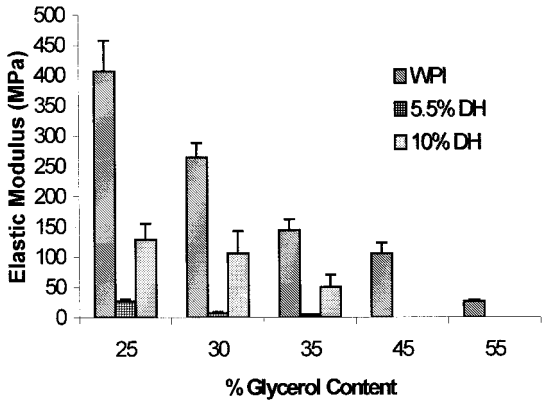

Figure 2. Effect of WPI DH on elastic modulus (EM) of edible films. Error bar shows standard deviation.

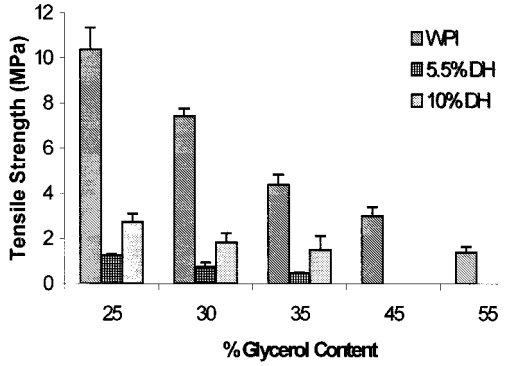

Figure 3. Effect of WPI DH on tensile strength (TS) of edible films. Error bar shows standard deviation.

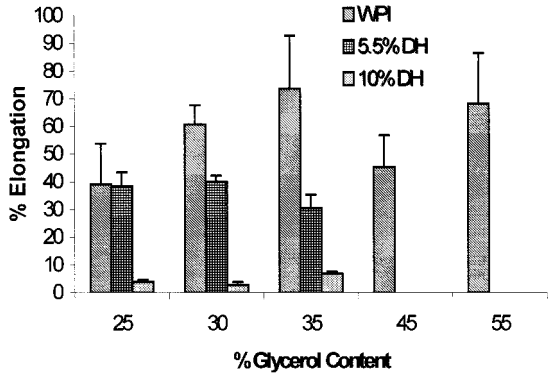

Figure 4. Effect of WPI DH on % elongation (%*E*) of edible films. Error bar shows standard deviation.

the effect on OP, the type of WPI had a significant effect on film EM, TS, and %*E* ($p \leq 0.05$), as shown in Figures 2−4. Hydrolyzed WPI films were more flexible (lower EM) and weaker and had smaller elongation (% strain at break) than did WPI at the same gly contents. Different DH gave different strength, with 10% DH WPI films being stiffer and stronger than 5.5% DH WPI films. Extent of M_w reduction cannot explain this situation, since 10% DH WPI has a smaller M_w than that of 5.5% DH WPI. The difference may be attributable to differences in the hydrolysis processes for the two materials. The 10% DH WPI films with 25% gly had equal EM but lower TS and %*E* compared to those of WPI films with 35% gly. These results are consistent with the shorter chains of hydrolyzed WPI films forming weaker protein network interactions between chains-compared to those of the longer chains of unhydrolyzed WPI.

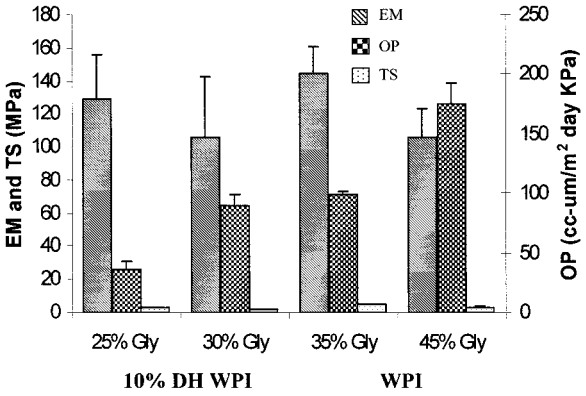

Figure 5. Comparison of EM, TS, and OP of WPI and 10% DH WPI films. Error bar shows standard deviation.

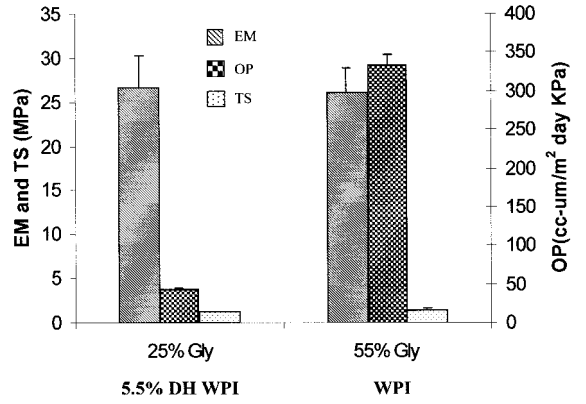

Figure 6. Comparison of EM, TS, and OP of WPI and 5.5% DH WPI films. Error bar shows standard deviation.

Comparison of Mechanical Properties and OP. Figure 5 shows that 25 and 30% gly in 10% DH WPI films had no significant difference ($p > 0.05$) in EM and TS when compared to 35 and 45% gly in WPI films, respectively. Moreover, 35% gly in 10% DH WPI films also had EM and TS values similar to those from 55% gly in WPI films (not shown). Films made of 25% gly in 5.5% DH WPI were comparable in EM and TS with films from 55% gly in WPI films (Figure 6). However, most hydrolyzed WPI films which had EM and TS values similar to those of unhydrolyzed WPI films had significantly less ($p \leq 0.05$) OP, with an especially large difference in 5.5% DH WPI films (Figures 5 and 6). These results show that hydrolyzed WPI films need less gly to achieve given EM and TS values and provide a better oxygen barrier.

Comparison of unhydrolyzed WPI and hydrolyzed WPI film OP with common synthetic polymer films is shown in Table 1. At the same test conditions, both unhydrolyzed WPI and hydrolyzed WPI achieve very good oxygen barrier films compared to those of low-density polyethylene (LDPE) and high-density polyethylene (HDPE), even at the higher gly content of unhydrolyzed WPI films. At low plasticizer levels, the WPI films have OP values similar to those of polyester and ethylene vinyl alcohol (EVOH) films at higher %RH (Salame, 1986; Hanlon, 1992; Miller and Krochta, 1997). Miller and Krochta (1997) explained that the globular nature (less linear structure) and large amino acid side groups of whey protein lead to decreased cohesive energy density and increased free volume compared to some synthetic polymers.

Table 1. Comparison of the OP of Unhydrolyzed, Hydrolyzed WPI, and Synthetic Polymer Films

film type	relative humidity[a]	permeability (cc μm/m^2 day KPa)
unhydrolyzed		
WPI/Gly = 3:1−0.8:1	50%	41.3−333.1
hydrolyzed		
5.5% DH WPI/Gly = 3:1−1.8:1	50%	42.2−111.9
10% DH WPI/Gly = 3:1−1.8:1	50%	35.6−89.1
synthetic polymers		
LDPE[b]	50%	1870
HDPE[c]	50%	427
polyester	50%	15.6
EVOH (70% VOH)[d]	0%	0.1
EVOH (70% VOH)	95%	12

[a] All tests done at 23 °C. [b] LDPE: low-density polyethylene. [c] HDPE: high-density polyethylene. [d] EVOH (70% VOH): ethylene vinyl alcohol polymer (70% vinyl alcohol)

CONCLUSIONS

Hydrolyzed WPI makes good films with OP values similar to but with more flexibility than WPI films at the same gly content. Correspondingly, the use of hydrolyzed WPI can reduce the gly amount needed to obtain desirable film flexibility, with resulting improvement in the film oxygen barrier. Consequently, hydrolyzed WPI films and coatings can be used to protect foods from oxygen while minimizing plasticizer usage and reducing the allergenicity of the whey protein.

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