Water Vapor Permeability and Solubility of Films from Hydrolyzed Whey Protein

R. SOTHORNVIT AND J.M. KROCHTA

ABSTRACT: The effects of whey protein hydrolysis on film water vapor permeability (WVP) and solubility at 3 plasticizer levels were studied. Little or no significant difference ($p > 0.05$) appeared for film WVP between unhydrolyzed whey protein isolate (WPI), 5.5% degree of hydrolysis (DH) WPI and 10% DH WPI films at comparable plasticizer contents. However, increase in glycerol (gly) content significantly increased film WVP. Thus, reduction in WPI molecular weight (MW) through hydrolysis may be a better approach to improving film flexibility than addition of plasticizer. Both 5.5% and 10% DH WPI had significantly different ($p \leq 0.05$) film solubility compared to unhydrolyzed WPI. Soluble Protein (SP) and total soluble matter (TSM) of hydrolyzed WPI films were much higher than for unhydrolyzed WPI films.

Key Words: hydrolyzed whey protein, permeability, solubility

Introduction

WORK ON INVESTIGATING THE POSSIBILITY OF USING EDIBLE polymer films and coatings on food systems has been conducted in order to improve food quality, add value to the edible polymer material and reduce synthetic packaging materials (Donhowe and Fennema 1993; Krochta 1997a). Edible films and coatings have shown potential for controlling transfer of moisture, oxygen, lipid, aroma, and flavor compounds in food systems, with resulting increase in food quality and shelf life (Donhowe and Fennema 1993; Krochta 1997b; Krochta and De Mulder-Johnston 1997c). Most research has focused on film formation and film properties. However, much research is still needed to better understand the effect of film structure on film properties.

Whey protein has been shown to make transparent films with good oxygen and aroma barrier properties (McHugh and others 1994; Mate and Krochta 1996a; Mate and Krochta 1996b; Miller and Krochta 1997). However, such films are quite brittle due to extensive intermolecular forces involving protein chain-to-chain interaction. One approach to overcome such film brittleness is addition of food grade plasticizers to the film formulation. Plasticizers reduce intermolecular forces along the whey protein polymer chains, with resulting increase in the mobility of polymer chains and more flexible films (Banker 1966). However, plasticizers not only improve the mechanical properties of films but also increase the film permeability (Gontard and others 1993). Increasing permeability is undesirable for food quality, so there is a need to minimize the use of plasticizers.

Another possible approach to reducing intermolecular forces along the whey protein polymer chains is to reduce polymer molecular weight (MW) by using whey protein isolate (WPI) with some degree of hydrolysis (DH), with resulting increase in polymer chain end groups and polymer free volume (Sears and Darby 1982). Reducing polymer MW may decrease the amount of added plasticizer needed in films; consequently, it may minimize permeability of films while producing needed film flexibility. Moreover, it has been known that using hydrolyzed WPI help reduce the allergenicity of milk protein, which is attributable to the globular nature of the protein (Nielsen 1997).

The objective of this study was to determine the effect of reduction of WPI MW on film WVP. If little or no increase in film permeability were to occur, reduction in whey protein MW could be a better approach to increasing film flexibility than addition of plasticizers. Effect of whey protein MW on film solubility was also determined.

Results and Discussion

Whey protein hydrolysis effects

Hydrolyzed WPI materials used in this study had 5.5% and 10% DH. DH is defined as the percentage of peptide bonds cleaved (Adler-Nissen 1979). As with WPI, hydrolyzed WPI could not form flexible stand-alone films without plasticizer addition. Hydrolyzed WPI films still needed plasticizer to reduce intermolecular forces between polymer chains by forming hydrogen bonds with the short chains of the WPI hydrolysates. However, they required a smaller amount of plasticizer than WPI films. Larger amounts of added plasticizer caused film stickiness and difficulty in peeling films from the casting plates, especially with 10% DH WPI films.

WVP. WVP of WPI films was slightly different from that of 10% DH WPI films ($p \leq 0.05$) at 25% glycerol (gly) content (Fig.1). However, there was no significant difference between WVP of WPI and 5.5% DH WPI films ($p > 0.05$). Furthermore, there was no significant difference found between unhydrolyzed WPI, 5.5% DH WPI, and 10% DH WPI film WVP at 30% and 35% gly content. These results are consistent with the results of Perez-Gago and others (1999) on WVP between native and denatured WPI films and with the results of Mate and Krochta (1996a) on WVP between WPI and β-lactoglobulin films, which showed that whey protein composition and structure had no effect on WVP. Thus, the effect of WPI hydrolysis on WVP does not show the same trend as added plasticizer on WVP.

Solubility. Film water solubility affects film use. For example, films on high-moisture foods must be insoluble, while films for water soluble pouches must be readily soluble. Solubility is an important protein functional property. Generally, protein solubility increases with an increase in DH, because of the reduction of protein MW resulting in increase of the number of polar groups. The same trend was found for WPI films. Whey protein MW had a significant effect on the percentage of total soluble matter (% TSM) and percentage of film soluble protein (% SP) ($p \leq 0.05$) (Fig. 2 and 3). Whey protein isolate films maintained their integrity in water, whereas 5.5%-DH WPI films completely

dissolved. The 10%-DH WPI films maintained their integrity during immersion, contrasted with 5.5%-DH WPI films, which completely dissolved. This is consistent with the finding that 10%-DH WPI films were stronger and had larger elastic modulus than 5.5%-DH WPI films.

The 10%-DH WPI films gave higher % TSM than WPI films, but less than that of 5.5%-DH WPI films. This might be due to the different enzyme systems, pH, treatment conditions, and so forth used to produce the 2 different hydrolyzed WPI products. According to the product description brochure, the 10%-DH WPI was obtained using enzymatic hydrolysis (protease enzymes) that were food grade GRAS (Generally Recognized As Safe). The 5.5%-DH WPI gave the highest % SP (64% to 75%) following by 10%-DH WPI films (30% to 34%) and WPI films (2.8% to 3.2%). These results are consistent with the % TSM results. Hydrolyzed WPI has increased solubility because the reduction of peptide bonds increases the NH_3^+ and COO^- content in the protein (Nielsen 1997). Thus, changes in properties due to hydrolysis of peptide bonds include decrease in MW, destruction of globular protein structure, and increase in solubility (Nielsen 1997).

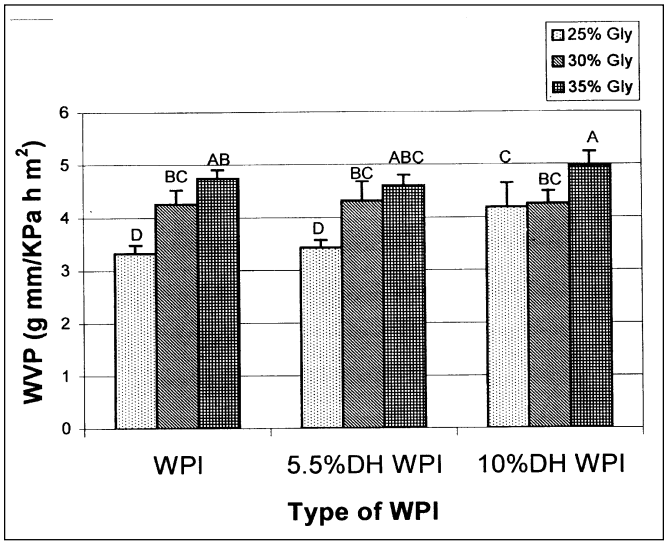

Fig. 1—Effect of whey protein hydrolysis and gly content on WVP of edible films. Statistical analyses for the effect of WPI types were performed separately for each plasticizer level using a Duncan's multiple range test (number of replication = 3). Columns with different letters are significantly different at $p \leq 0.05$. Error bar shows standard deviation.

Glycerol content effects

Plasticizer must be added to form intact films from either WPI or hydrolyzed WPI. To compare the effect of gly content in all types of WPI, the range of gly content selected was 25% to 35% (w/w). This was because at least 25% gly was needed to reduce brittleness and obtain intact films from WPI (Mahmoud and Savello 1993; McHugh and others 1994) while not more than 35% gly could be added to obtain films from 5.5%-DH WPI, and 10%-DH WPI before obtaining overly-sticky soft films.

WVP. Amount of plasticizer produced a significant difference in film WVP. Increasing gly amount gave significant increase in film WVP here (Fig. 1) and in most edible films, such as fish myofibrillar protein-based films (Cuq and others 1997), zein films (Koelsch 1994), whey protein films (McHugh and others 1994), wheat gluten films (Gontard and others 1993), and also in polysaccharide films (Schultz 1949). This has been well known, since plasticizer reduces intermolecular forces along protein chains and increases the polymer free volume. Therefore, there is greater space for water and other molecules to migrate. Moreover, hydrophilic plasticizer is compatible with hydrophilic film-forming material, such as protein, and enhances sorption of polar molecules, such as water.

Solubility. Plasticizer content did not have a significant effect on % TSM and % SP ($p > 0.05$) (Fig. 2 and 3). In general, this result was consistent with no significant difference on TSM and SP

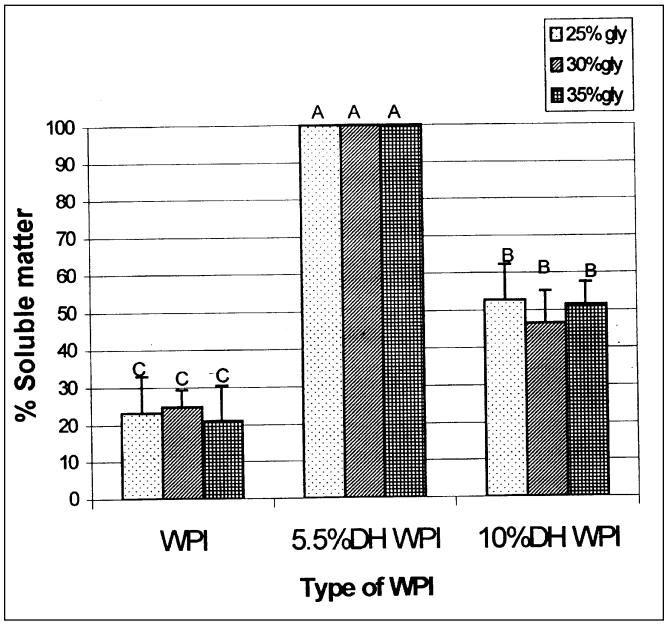

Fig. 2—Effect of whey protein hydrolysis and gly content on % total soluble matter of edible films. Statistical analyses for the effect of WPI types were performed separately for each plasticizer level using a Duncan's multiple range test (number of replication = 3). Columns with different letters are significantly different at $p \leq 0.05$. Error bar shows standard deviation.

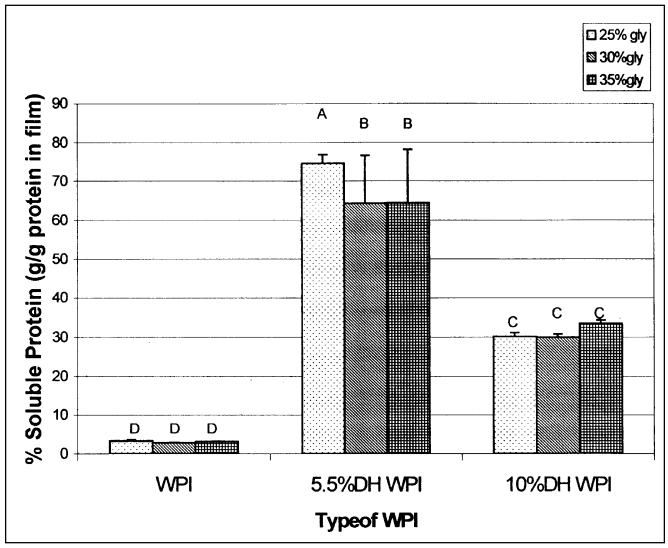

Fig. 3—Effect of whey protein hydrolysis and Gly content on % soluble protein of edible films. Statistical analyses for the effect of WPI types were performed separately for each plasticizer level using a Duncan's multiple range test (number of replication = 3). Columns with different letters are significantly different at $p \leq 0.05$. Error bar shows standard deviation.

of soy protein films in the range of 17% to 23% gly content (Stuchell and Krochta 1994). The 25% to 35% gly content might be too small a range of gly content to see a plasticizer effect. Cuq and others (1997) found that an increase in plasticizer content produced a linear increase in water-soluble matter content in fish myofibrillar protein films. However, over the same gly level range of our study, they found no significant difference in solubility. Soluble protein is affected by temperature, pH, presence of other solutes and salts. TSM of WPI films represents only the gly amount (McHugh and Krochta 1994). However, hydrolyzed WPI has soluble protein components, which increase the total soluble matter.

Conclusions

HYDROLYZED WPI MAKES GOOD FILMS WITH GREATER SOLUBILity than WPI films. This study also showed that whey protein MW has little or no effect on film WVP. Thus, use of hydrolyzed WPI can likely reduce the plasticizer content required to impart desired film flexibility and can thus minimize permeability of WPI films. Further study of whey protein MW on film mechanical properties along with other permeability properties (for example, oxygen) is appropriate. Since hydrolyzed WPI reduces the allergenicity of milk protein (New Zealand Milk Products Inc. 1997), hydrolyzed WPI coatings may be acceptable on nuts, fruits, and other foods for consumers with whey protein allergy.

Materials and Methods

Materials

BiPro WPI (97.7% protein dry basis (db) and 5.5% DH WPI (96.3% protein db and 5.5% DH) supplied by Davisco Foods International (Le Sueur, Minn., U.S.A.) and 10% DH WPI (90.1% protein db, 10% DH and MW = 1100 Daltons as declared by company) supplied by New Zealand Milk Products were used to make films. Both hydrolyzed products are produced enzymatically. Glycerol (gly) as a plasticizer was purchased from Fisher Scientific Inc. (Fair Lawn, N.J., U.S.A.). Bicinchonic acid protein assay kit, bovine serum albumin (BSA), and potassium sorbate as reagents to determine protein solubility were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

Film formation

Aqueous solutions of either WPI, 5.5%-DH WPI or 10%-DH WPI (10% w/w) 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 (RT) and degassed by applying vacuum to remove dissolved air, followed by adding 25%, 30%, or 35% (w/w) of gly plasticizer and then degassing again. Films were cast by pipetting solutions onto 14.7-cm internal diameter, rimmed, smooth high-density polyethylene (HDPE) plates. They were placed on a leveled surface at RT (23 °C and 35% ± 5% RH) until dried films could be released intact from plates. Whey protein solutions with 3 g total solids per plate were used to reduce thickness variation among films. Three replications and 4 repeated observations were used to determine each property.

Film thickness

Film thickness was measured with a micrometer (No. 7326, Mitutoyo Manufacturing Co. Ltd., Tokyo, Japan) to the nearest 0.0001 in (0.00254 mm) around the film testing area at 5 random positions. An average of the 5 values of film thickness was used to determine WVP for each film replicate.

Water Vapor Permeability (WVP) measurement

The gravimetric Modified Cup Method based on ASTM E96-92 (McHugh and others 1993) was used to determine WVP. Six ml of deionized water was pipetted into test cups made of polymethylmethacrylate (Plexiglas) with external dimension of 8.2-cm diam, 1.25-cm height, and 21.56-cm^3 inner volume. Films without pinholes and any defects were placed in between the cup and the ring cover of each cup coated with silicon sealant (high vacuum grease, Dow Corning Midland, Mich., U.S.A.) and held with 4 screws around the cup circumference. After that, the cups were placed in constant RH cabinets (0% RH using anhydrous calcium sulphate desiccant from W.A. Hammond Drierite Co. (Xenia, Ohio, U.S.A., inside cabinet), located in a controlled temperature room at 25 °C. Once steady-state moisture transfer was obtained, weights were taken at 2-h intervals. The WVP of film was calculated by multiplying the steady state water vapor transmission rate by the film thickness and dividing by the water vapor partial pressure difference across the films.

$$WVP = \frac{WVTR * thickness}{(p_{A1} - p_{A2})}$$

where WVTR = water vapor transmission rate and p_{A1} and p_{A2} = water vapor partial pressure inside and outside the cup, respectively

Film solubility measurement

Protein Solubility. A piece of film sized 7.5 mm × 15.0 mm was cut and dried in a vacuum oven at 70 °C and 5 psi for 24 h and then weighed to obtain the initial film dry weight. The piece of film was then placed into a test tube with 10 ml deionized water and 0.01% potassium sorbate to prevent microbial growth. This small amount of potassium sorbate did not affect solubility of films. The test tube was capped and shaken slowly on a shaking platform for 24 h at RT. Film protein solubility was determined by using the bicinchoninic acid (BCA) protein assay according to Smith and others (1985). Supernatant was pipetted at 200 μl into the protein determination reagent (1 part of 4% copper (II) sulfate pentahydrate solution and 50 parts of BCA solution), mixed, heated in a water bath at 37 °C for 30 min and then cooled to RT. Absorbance of the mixture was read at 562 nm using a Shimadzu UV-Vis Recording Spectrophotometer UV-160 A (Shimadzu Scientific Instrument Corp., Columbia, Md., U.S.A.). A standard curve using BSA was obtained to determine protein concentration.

The percentage of film soluble protein (% SP) was calculated by dividing the weight of soluble protein in 10 ml of film-immersing solution by the initial dry weight of protein in the film piece. The initial dry weight of protein in the film piece was computed by multiplying the initial dry weight of the film by the ratio of WPI weight to total solid weight in the film and by the fraction of whey protein in WPI material. Thus:

$$\%SP = \left(\frac{wt\ of\ protein\ in\ 10\ ml\ solution}{(initial\ dry\ film\ wt)(fraction\ of\ protein\ in\ WPI)\left(\frac{WPI\ wt\ in\ film}{total\ solid\ wt\ in\ film}\right)} \right) * 100$$

Film solubility (total soluble matter). The remaining film after immersing in the solution was dried in the vacuum oven

at 70 °C and 5 psi for 24 h to determine the film final dry weight. The percentage of total soluble matter (% TSM) of the film was calculated from a following equation

$$\%\ TSM = \left(\frac{initial\ \ dry\ \ film\ \ wt - final\ \ dry\ \ film\ \ wt}{initial\ \ dry\ \ film\ \ wt} \right) * 100$$

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 the significant treatments at 95% confidence interval.

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