Mechanical Properties and Water Vapor Permeability of Edible Films from Whey Protein Isolate and *N*-Ethylmaleimide or Cysteine

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The role of sulfhydryl/disulfide interchange in determining the water vapor permeability (WVP) and mechanical properties of edible films from whey protein isolate (WPI) was investigated. Nearly total inhibition of sulfhydryl/disulfide interchange by the sulfhydryl blocking agent *N*-ethylmaleimide (NEM) reduced protein solubility by 50%, but had no effect on WVP, Young's modulus, yield stress, or breaking stress. Breaking strain was reduced significantly at high levels of added NEM. Reduction of disulfide bonds with cysteine had no effect on WVP. The effects of hydrogen bonding far outweigh those of disulfide bonding in WPI films.

Keywords: *Edible films; biodegradable films; elastic modulus; tensile stress; sulfhydryl/disulfide interchange; thiol/disulfide interchange*

INTRODUCTION

Whey protein isolate (WPI) can be formed into edible films that are excellent barriers to the transport of oxygen and carbon dioxide, though moderate barriers to the transport of moisture (Krochta, 1992). WPI films are brittle and must be plasticized with a compound such as glycerol, sorbitol, or poly(ethylene glycol) (Guilbert, 1986). These plasticizers are thought to disrupt hydrogen bonding between neighboring protein strands, so that interchain attractive forces are reduced and chain mobility is increased (Kester and Fennema, 1986; Guilbert, 1986). The addition of plasticizers adversely affects the barrier properties of the film (McHugh and Krochta, 1994), and there is a need to find more effective plasticizers for proteinaceous, edible films. However, there is only limited understanding of the forces that affect the structure of such films. In addition to hydrogen bonds, there are also disulfide bonds in films based on WPI (McHugh and Krochta, 1994). The purpose of this study was to determine the importance of disulfide bonds in edible films from WPI.

The major component proteins of WPI are β-lactoglobulin (β-Lg), α-lactalbumin, bovine serum albumin (BSA), and a variety of immunoglobulins (Kinsella and Whitehead, 1989). Free sulfhydryl groups that are normally occluded within β-Lg and BSA can be exposed by heating, treatment with alkali (Mulvihill et al., 1991) or urea (Xiong and Kinsella, 1990), and by adsorption at an interface (Monahan et al., 1993; McClements et al., 1993). At neutral or alkaline pH, free sulfhydryls rapidly interchange with existing disulfide bonds to generate new inter- and intramolecular disulfide bonds in a chain reaction that regenerates free sulfhydryl (Jensen, 1959):

$$R_1S{-}SR_2 + R_3SH \rightarrow R_1SH + R_2S{-}SR_3$$

All the component proteins of WPI contain disulfide bonds, so the possibilities for polymerization via sulfhydryl/disulfide interchange are numerous.

The role of sulfhydryl/disulfide interchange in the gelation of whey proteins has been studied. Although gelation is a complex process involving all the different types of bonding known to stabilize proteins (Grinberg et al., 1992; Mangino, 1992), sulfhydryl/disulfide interchange is a key component in the association of whey proteins. The strength of whey protein gels is greatly reduced when free sulfhydryl groups are blocked by *N*-ethylmaleimide (NEM) (Zirbel and Kinsella, 1988; Mulvihill et al., 1991) or when disulfide bonds are reduced by cysteine (Schmidt et al., 1979). Given the importance of disulfide bonds in gelation, these bonds might also be expected to be important in film formation. In the present study, NEM and cysteine were used to determine the role of sulfhydryl/disulfide interchange in edible films based on WPI.

MATERIALS AND METHODS

Materials. WPI (>95% protein on a dry weight basis) was obtained from Le Sueur Isolates, Le Sueur, MN. Sodium azide, sodium dodecyl sulfate (SDS), *N*-ethylmaleimide, and cysteine (all >99%) were obtained from Sigma Chemical Company, St. Louis, MO. Glycerol (>99%) was obtained from Fisher Scientific, Fair Lawn, NJ. Reagent grade tris(hydroxymethyl)methylamine (Tris) and ethylenediamine tetraacetic acid, disodium salt (EDTA) were obtained from BDH Chemicals Ltd., Poole, U.K. Urea and glycine (both >99%) were obtained from Merck, Darmstadt, Germany.

Film-Forming Solution. The WPI used in this work contained ~68 wt % β-Lg (Mate and Krochta, 1994). Using this figure and compositional data from Kinsella et al. (1989), the average molecular weight of WPI was calculated as ~30 400 daltons. Films were formed from a 10 wt % solution of WPI, with sodium azide (0.02 wt %) as a preservative. This solution was estimated to be ~3.8 mM in free sulfhydryl groups and 14.3 mM in cystine residues.

Films with Added NEM. To aliquots (275 g each) of WPI solution, different amounts of NEM were added; the amounts were calculated to be sufficient to react (in theory) with 0–100% of the free sulfhydryl groups. The solutions were deaerated and heated, with stirring, to 90 °C. Under these

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conditions, NEM binds rapidly and specifically to free sulfhydryls (Xiong and Kinsella, 1990). After 30 min, the solutions were cooled to room temperature and sufficient glycerol was added to give a WPI:glycerol mass ratio of 3:1.

Films with Added Cysteine. To aliquots (27.27 g each) of WPI solution, different amounts of cysteine were added; the amounts were sufficient to reduce (in theory) 0−150% of the disulfide bonds. The mixtures were deaerated, heated, and plasticized in the way just described. Films were also prepared from solutions containing cysteine that had been added after the heating step was complete. The films with added cysteine were used only for tests of water vapor permeability.

Film Formation. Aliquots of film-forming solution (~20 g) were weighed onto smooth, circular, rimmed plates (14.7 cm i.d.; ultra high molecular weight, high density polyethylene) to obtain 3.0 g of solids per plate. The solutions were spread out with a bent glass rod to cover each plate. The solutions were left to dry into films overnight on level aluminum slabs, at room temperature.

Solubility and Sulfhydryl Content. Solubility of the protein in the films was determined by a modification of the method of Shimada and Cheftel (1989). Film samples containing ~0.1 g of protein were soaked in 20 mL of buffer (0.086 M Tris, 0.09 M glycine, EDTA, 8 M urea, 0.5 wt % SDS, pH 8) for 16 h at room temperature. The holding period at room temperature increased the solubilization of the protein in the film. The film was homogenized for 6 min with an UltraTurrax (IKA-works, Inc., Cincinnati, OH) homogenizer, and the homogenate was centrifuged for 30 min at 2000*g*. The protein content of the supernatant was determined from the absorbance at 280 nm as described by Shimada and Cheftel (1989). The free sulfhydryl content of the solubilized protein was determined by the method of Ellman (1959). Between three and six replicates were analyzed in each case.

Water Vapor Permeability (WVP). The WVP of each film was determined according to the WVP Correction Method of McHugh et al. which is described fully elsewhere (McHugh et al., 1993). Briefly, aliquots of distilled water were dispensed into shallow, flat-bottomed Plexiglass cups with wide rims. The rim of each cup was lightly smeared with high vacuum silicone grease, and then a sample of film was placed over the top of the cup and secured tightly in place by a sealing ring. The underside of the ring (i.e., the side that was in contact with the top of the film) was also coated with silicone grease, and the ring was held in place by four equally spaced brass screws. The cups were placed in chambers at 25 ± 2 °C and 0% RH. The air in the chambers was rapidly recirculated by a powerful fan. The WVP was determined by weighing the cups periodically to determine the loss of moisture through the film. Six replicates were examined for each NEM film formulation. Three replicates were examined for each cysteine film formulation. The thickness of each replicate was taken as the average of five measurements taken at random points on the film immediately after WVP determination with a micrometer (model 7326, Mitutoyo Corporation, Japan).

Mechanical Properties. Test pieces were punched out of films by a 25.4 × 152.4 mm (1″ × 6″) die in a hand-operated press. The pieces were equilibrated in a controlled environment room at 23 ± 2 °C and 65 ± 2% RH for at least 1 week before testing. The thickness of each piece was taken as the average of six measurements taken at random points on the equilibrated sample with the micrometer described above. Samples were tested on an Instron Universal Testing machine (model 1122) in a controlled environment room at 23 ± 2 °C and 65 ± 2% RH. A 222 N (50 lb) load cell with self-aligning grips and 25.4 × 50.8 mm (1″ × 2″) stainless steel faces (Instron Corporation) was used. The gauge length was 101.6 mm (4″) and the rate of grip separation was 5 mm min^{-1}. Tensile properties were calculated with Series IX Automated Materials Testing System software (Instron Corporation). Between 14 and 26 replicates were tested for each film formulation.

RESULTS AND DISCUSSION

Solubility of WPI Films with Added NEM. About 21% of the protein in the control film was soluble in the solubilizing buffer (Figure 1), which is consistent with the observation that WPI films are largely insoluble (McHugh and Krochta, 1994). The decrease in protein solubility at higher concentrations of NEM (Figure 1) was unexpected because sulfhydryl/disulfide interchange is known to be responsible for insolubility of protein powders such as soy (Hoshi et al., 1982). Possibly the prevention of sulfhydryl/disulfide interchange enables the protein to adopt conformations that result in increased hydrogen bonding in the film or that result in increased hydrophobicity. A follow-up experiment combining NEM and SDS to simultaneously inhibit sulfhydryl/disulfide interchange and disrupt hydrogen bonding would be needed to more fully explain the unexpected decrease in solubility seen here.

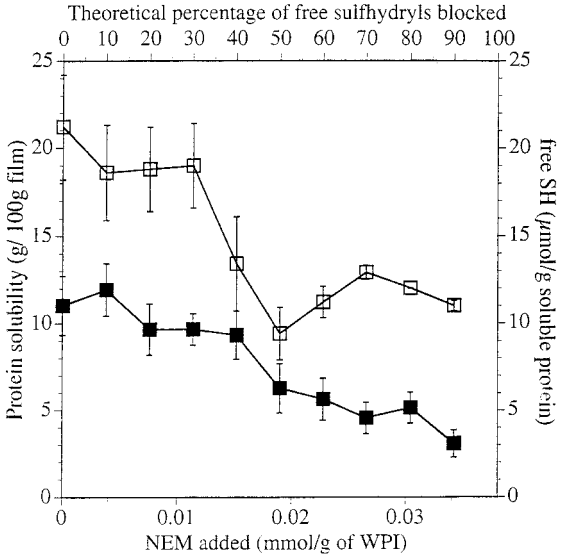

Figure 1. Solubility (□) and free sulfhydryl content (■) of WPI films containing different amounts of NEM. The error bars represent 1 standard deviation.

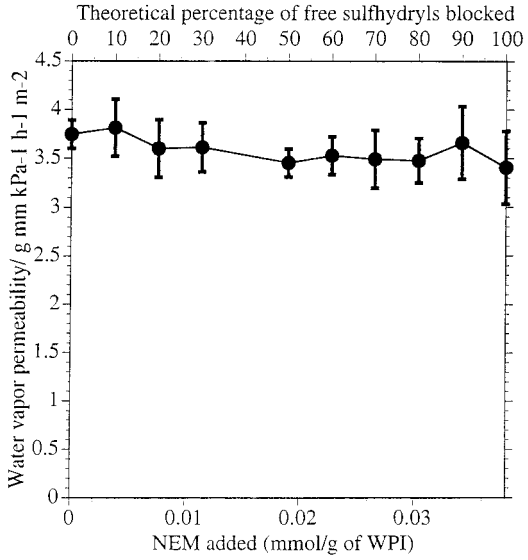

Figure 2. Water vapor permeability of WPI films containing different amounts of NEM. The error bars represent 1 standard deviation.

Fraction of Free Sulfhydryls Blocked. The measurable free sulfhydryl content of the films decreased by ~75% as the amount of added NEM increased (Figure 1). Although a decrease in measurable free sulfhydryls was to be expected, control films with no added NEM had a free sulfhydryl content of 11 μmol/g

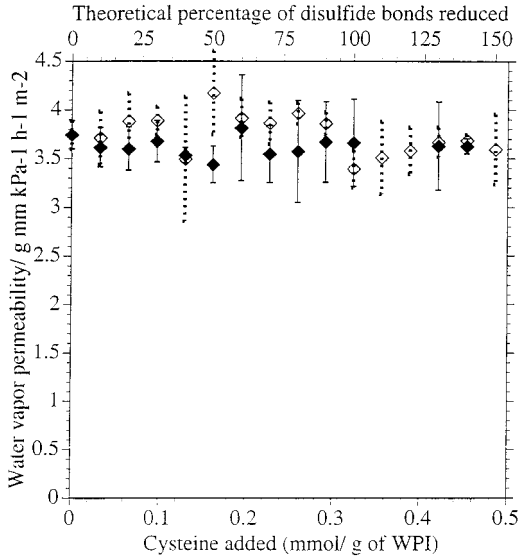

Figure 3. Water vapor permeability of WPI films containing different amounts of L-cysteine, added before (◆) or after (◇) heating. The error bars represent 1 standard deviation.

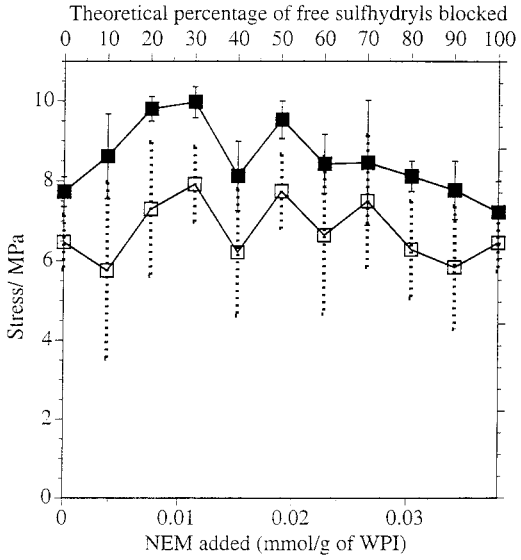

Figure 5. Breaking stress (□) and yield stress (■) of WPI films containing different amounts of NEM. The error bars represent 1 standard deviation.

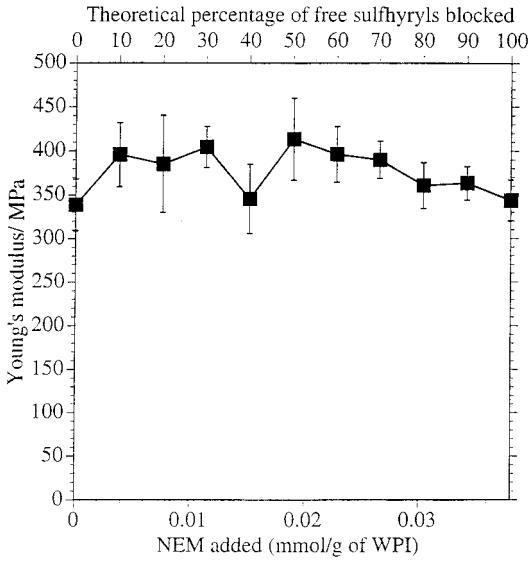

Figure 4. Young's modulus of WPI films containing different amounts of NEM. The error bars represent 1 standard deviation.

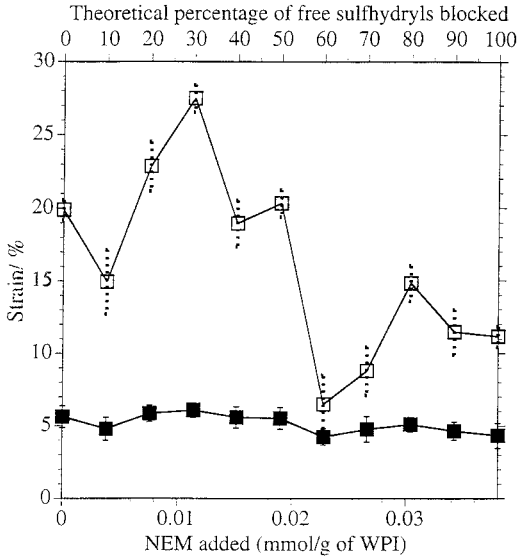

Figure 6. Breaking strain (□) and yield strain (■) of WPI films containing different amounts of NEM. The error bars represent 1 standard deviation.

protein. This value was rather lower than the 28 μmol/g (at 25 °C and pH 7.0) reported by Lee et al. (1992), and the 38 μmol/g implied by the free sulfhydryl content that was calculated earlier. The difference was probably due to difficulties in solubilizing the protein for analysis and oxidation of free sulfhydryls during film formation.

Water Vapor Permeability. There was no statistically significant trend in WVP as the fraction of free sulfhydryl groups that were blocked was increased (Figure 2), so sulfhydryl/disulfide interchange had no effect on the WVP. Cysteine had very little effect on WVP, whether added before or after heating (Figure 3). Some caution is necessary when considering Figure 3, because cysteine can interact with the disulfide bonds in proteins in a variety of ways (Koh et al., 1996), and under some experimental conditions it may have little effect on sulfhydryl/disulfide interchange. Nevertheless, it appears that disulfide bonds, whether intra- or intermolecular, play a very small role in determining the moisture barrier properties of films based on WPI. Although individual covalent bonds are much stronger than individual hydrogen bonds, there are clearly many more hydrogen bonds than disulfide bonds in WPI film.

Mechanical Properties. Blocking free sulfhydryls with NEM had little effect on Young's modulus (Figure 4), yield stress, and breaking stress (Figure 5). Yield strain declined slightly, and breaking strain declined significantly as the amount of added NEM increased (Figure 6). Thus, the addition of NEM did not make the films any weaker, but it did make them slightly less extensible. Again, this could be because the prevention of sulfhydryl/disulfide interchange promotes a change in protein conformation that leads to increased hydrogen bonding. This increased bonding could offset the loss in strength and increase in extensibility that would be expected from the suppression of intermolecular disulfide bonding.

Sulfhydryl/disulfide interchange does not play a significant role in determining the functional properties of films from WPI. This result suggests that the detailed understanding of intermolecular forces that exists for gelation and aggregation can only partly

explain the behavior of edible films. Thus, edible films cannot simply be regarded as dried gels; the relative importance of the stabilizing forces, such as hydrogen bonding, hydrophobic bonding, and ionic bonding, is different and largely unknown. Research into improved proteinaceous, edible films should therefore concentrate on determining the balance of these forces, to develop a better strategy for plasticization.

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