Denaturation Time and Temperature Effects on Solubility, Tensile Properties, and Oxygen Permeability of Whey Protein Edible Films

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ABSTRACT: Whey protein film solubility decreased as film-forming solution heating time and temperature increased. No differences were observed in solubility between films soaked in water at 25 °C for 24 h and 100 °C for 4 min. However, the degree of swelling was significantly larger for films soaked at 100 °C. Films became stiffer, stronger and more stretchable as film-forming solution time and temperature were increased. Oxygen permeability (OP) was lower for films made from heat-denatured whey protein than for films made from native whey protein. Results suggest that an increase in covalent cross-linking, as heat denaturation of the whey protein increases, is accountable for film water insolubility, higher tensile properties and lower OP.

Keywords: whey protein, heat denaturation, oxygen permeability, film solubility, tensile properties.

Introduction

EDIBLE FILMS AND COATINGS CAN PREVENT QUALITY CHANGES in foods by acting as barriers to moisture, oxygen, oil, and aroma migration between adjacent food components and/or between the food and the environment. Edible films and coatings can also carry food ingredients, improve mechanical integrity of foods and reduce the packaging material required for food products. The techniques of film formation, properties and applications of films and coatings based on lipids, polysaccharides and proteins, by themselves or in combination, have been reviewed (Guilbert 1986; Kester and Fennema 1986; Krochta and others 1994; Krochta and De Mulder-Johnston 1997; Debeaufort and others 1998).

Whey protein has excellent nutritional and functional properties and the ability to form films. Whey protein has been shown to produce transparent, bland, flexible, water-based edible films with excellent oxygen, aroma and oil barrier properties at low relative humidity (McHugh and Krochta 1994a; Miller and Krochta 1997; De Mulder-Johnston 1999). On the other hand, whey protein films provide a poor moisture barrier. However, incorporation of lipids reduces their water vapor permeability (WVP) (McHugh and Krochta 1994b; Shellhammer and Krochta 1997; Pérez-Gago and Krochta 1999).

The characteristics of protein-based films are determined by the nature of protein-protein interactions. Film-forming ability may be influenced by amino acid composition, distribution and polarity, ionic cross-links between amino and carboxyl groups, hydrogen bonding groups, and intramolecular and intermolecular S-S bonds (Gennadios and Weller 1991).

Native whey proteins are globular proteins containing most of the hydrophobic and SH groups hidden in the interior of the molecule. Formation of whey protein films has mainly involved heat denaturation of whey proteins in aqueous solutions. Heating modifies the 3-dimensional structure of the protein, exposing internal SH and hydrophobic groups (Shimada and Cheftel 1989), which promote intermolecular S-S and hydrophobic bonding upon drying (McHugh and Krochta 1994b). McHugh and Krochta (1994a) studied the optimization of whey protein film-forming conditions and found that, for the film formulations studied, heat treatment was necessary (for example, 90 °C for 30 min) for the formation of intact whey-protein-based edible films. These films were characterized by their water insolubility, which can be beneficial in maintaining film and food integrity. Pérez-Gago and others (1999) found that films from native whey protein (that is, whey protein which has not undergone heat treatment) could also be formed. Since native whey proteins maintain their globular structure with most of the hydrophobic and SH groups buried in the interior of the molecule, native protein films have a structure in which cohesion relies mainly on hydrogen bonding. In contrast, the intermolecular forces that promote cohesion in heat-denatured films also involve intermolecular S-S and hydrophobic bonds among the unfolded protein strands. Both native and heat-denatured whey protein films are transparent, having similar water vapor permeabilities; however, they possess different solubility and mechanical properties. The unfolded structure of heat-denatured whey proteins and the covalent S-S bonding during drying lead to film insolubility in water and produce films that are stronger and which can withstand higher deformations. The structure and low energy interactions of native whey protein films result in solubility in water and poor mechanical properties compared to heat-denatured whey protein films (Pérez-Gago and others 1999).

Solubility in water is an important property of edible films. Potential applications may require water insolubility to enhance product integrity and water resistance. However, in some cases film water solubility before consumption of the product might be beneficial. The degree of protein denaturation and unfolding as heating time and temperature are increased probably affects the degree and nature of protein-protein cross-linking and, as a consequence, the solubility and mechanical properties of the films. A more comprehensive understanding of film properties, as affected by heat-denaturation conditions is necessary to allow for optimization of film forming conditions depending on the desired film properties. In addition, film solubility is usually measured by soaking the film in water at room conditions for 24 h. No research has been done studying film solubility in boiling water.

Film solubility might be affected by the temperature of the water, depending on the degree and nature of protein-protein interactions. Furthermore, previous results showed no significant difference between WVP of native and heat denatured WPI films, but no results are available for oxygen permeability (OP). Our objective was to study the effect of film-forming solution heating time and temperature on solubility, film swelling, tensile properties and OP of whey protein films.

Materials and Methods

Materials

Whey protein isolate (WPI) (~ 97.7% dry basis protein) was supplied by Davisco Foods International (Le Sueur, Minn., U.S.A.). Glycerol (Gly) (Fisher Scientific Inc., Fair Lawn, N.J., U.S.A.) was added as a plasticizer to all film-forming solutions. Bicinchoninic Acid Protein Assay Kit and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.).

Film Formation

Aqueous 200mL solutions of 10% (w/w) WPI were prepared and degassed under vacuum. WPI solutions were heat-denatured in a water bath held at either 70, 80, 90 or 100 °C for 5, 10, 15 or 20 min. Temperatures of the heated solutions were recorded as soon as the solutions were taken from the water bath. Results are shown in Table 1. Heated solutions were then cooled to room temperature to avoid further denaturation. Gly was added to all film-forming solutions as a plasticizer in a 70/30 ratio of WPI to Gly. Before being cast, the final solutions were degassed under vacuum to remove any dissolved air. Native films were also prepared and underwent the same film-forming process, with the exception of the heat treatment.

Films were cast by pipetting whey protein solution onto rimmed, smooth high-density polyethylene (HDPE) plates resting on a leveled granite slab. WPI solutions were applied at 3 g of total solids onto a 15.5 cm internal diameter plate to prepare films for solubility and OP testing, and at 10.8 g total solids onto square 22.5 cm by 30.2 cm plates for tensile properties determination. This way, thickness variations between treatments were minimized. Three films were prepared for each treatment corresponding to 3 different replicates. The films were allowed to dry at room temperature over 24 h.

Film Thickness

Thicknesses of the films were measured with a caliper micrometer (No. 7326, Mitutoyo Manufacturing Co. Ltd., Japan) at 6 random positions of the film, following OP and preceding tensile tests. OP and mechanical properties were calculated using the average thickness.

Film Protein Solubility

Three films of each treatment were cast corresponding to 3 different replicates. Four rectangular pieces, measuring 7.5 mm by 15.0 mm, were cut from each film. Those pieces were dried in a vacuum oven at 70 °C and 34.5 kPa for 24 h (until constant weight) to determine the initial dry weight of the film. The samples were weighed to the nearest 0.00001 g. Solubility tests were done at 2 different conditions: (1) films soaked in water at ambient conditions (~ 25 °C) for 24 h; and (2) films soaked in water at 100 °C for 4 min. Two of the samples were placed into test tubes with 7 ml of deionized water at ambient temperature. The tubes were capped and placed on a rotating platform at 250 rpm for 24 h. The other 2 samples were placed into test tubes with 7 ml of deionized water previously heated to 100 °C. The tubes were capped and placed in a water bath held at 100 °C for 4 min. After heating, tubes were cooled down to room temperature in an ice bath. The soluble protein of films in water was determined by using the bicinchoninic acid (BCA) protein assay (Smith and others 1985). Samples from the test tubes were added to the protein determination reagent, made by combining 1 part 4% copper (II) sulfate pentahydrate solution with 50 parts BCA solution, vortexing and heating to 37 °C for 30 min in a water bath. After cooling down to room temperature, absorbances of mixtures were read at 562 nm using a Shimadzu UV-Vis Recording Spectrophotometer UV-160A (Shimadzu Scientific Instruments Corp., Columbia, MD). Protein concentrations were calculated from a standard curve obtained using bovine serum albumin.

Table 1—Temperature of WPI solution (°C) after heat denaturation at selected water- bath temperatures and times

	Water Bath Temperature (°C)			
Time (min)	70	80	90	100
5	59 ± 2	66 ± 2	72 ± 1	76 ± 2
10	62 ± 2	71 ± 2	79 ± 3	87 ± 1
15	65 ± 1	72 ± 2	81 ± 2	91 ± 1
20	66 ± 2	73 ± 2	82 ± 2	91 ± 1

Temperatures are mean values of 3 replicates.
Unshadowed and shadowed areas separate denaturation conditions that form water-soluble (unshadowed area) compared to insoluble (shadowed area) WPI films.

The percentage of soluble protein (%SP) in the film was calculated by dividing the weight (wt) of soluble protein in the 7 ml of film soaking solution by the initial dry weight of protein in the film piece. The initial dry weight of protein in the film was calculated from the initial dry weight of the film, taking into account both the ratio of WPI:total solids in the film and the percent protein in the WPI powder (97.7%). The following equation was used:

$$\%SP = \left(\frac{Wt\ protein\ in\ 7mL\ solution}{Initial\ wt\ film * 0.977 * Ratio\ of\ WPI:Total\ solids\ in\ film} \right) * 100\% \quad (1)$$

Film Solubility (Total Soluble Matter)

The remaining pieces of film right after soaking for 24 h at ambient conditions or 4 min at 100 °C were poured onto Whatman #1 qualitative filter paper (previously dried and weighed under similar conditions) and dried again in the vacuum oven at 70 °C and 34.5 kPa for 24 h to obtain the final dry weight of the film. The percentage of total soluble matter (%SM) of the films was calculated using the formula below:

$$\%SM = \left(\frac{Initial\ dry\ weight - Final\ dry\ weight}{Initial\ dry\ weight} \right) * 100\% \quad (2)$$

Film Swelling

Length and width of the samples were measured to the nearest mm before and after soaking the films in water. Swelling of the films after soaking was calculated using the formula below:

$$\%Swelling = \left(\frac{Final\ film\ area - Initial\ film\ area}{Initial\ film\ area}\right) * 100\% \quad (3)$$

Tensile Properties Determination

Dried films were conditioned at 100% RH in a chamber for a few hours. This preconditioning provided ease of handling and cutting of the films. Test pieces of film were cut using a striking die (The Right Image, Sacramento, Calif., U.S.A.). The cut film samples had a rectangular center section measuring 15 mm wide by 100 mm long, flaring to 25 mm by 35 mm square sections on each end, which allowed for a greater grip area. All the film strips were equilibrated for at least 48 h to 53% RH in a cabinet using magnesium nitrate (Fisher Scientific Inc., Fair Lawn, N.J., U.S.A.) saturated solution at room temperature (23 ± 2 °C).

Tensile measurements (tensile strength (TS), Young's Modulus (YM) and % elongation (%E)) were performed following the procedure outlined in ASTM method D882-97 (ASTM 1997). The ends of the equilibrated film strips were mounted and clamped with pneumatic grips on a Universal Testing Machine (Model 1122, Instron Corp., Canton, Mass., U.S.A.) with a 500 kg load cell. The initial gauge length was set to 115 mm and films were stretched using a crosshead speed of 50 mm/min. Testing conditions were controlled throughout the measurements and held constant at 23 ± 2 °C and 50 ± 5% RH. Tensile properties were calculated from the plot of stress (tensile force/initial cross-sectional area) versus strain (extension as a fraction of the original length), using Series IX Automated Materials Testing System Software (Instron). Mechanical properties reported are maximum tensile stress (MPa), elongation at break (%) and Young's Modulus (MPa).

Oxygen Permeability Measurements

Oxygen permeability (OP) of plasticized native (that is, whey protein which has not undergone heat treatment) and heat-denatured films (for example, in water bath held at 90 °C for 30 min) was measured at 23 °C and 50 ± 1% RH using an Ox-Tran 2/20 modular system (Modern Control, Inc., Minneapolis, Minn., U.S.A.) according to the American Society of Testing and Materials Standard Method D3985 (ASTM 1995). Films were placed on a stainless steel mask with an open testing area of 5 cm^2. Masked films were placed into the test cell and exposed to 98% N_2 + 2% H_2 flow on one side and pure oxygen flow on the other. OP was calculated by dividing the oxygen transmission rate by the difference in oxygen partial pressure between both sides of the film (1 atm) and multiplying by the average film thickness, measured at 4 random places. Three replicates of each film were evaluated.

Statistical Analysis

Statistical analysis was performed using SAS software (SAS Institute Inc., 1996). Duncan's multiple comparison test ($p \leq 0.01$) was used to determine significance of differences between means.

Results and Discussion

Film Protein Solubility

Solubility in water is an important property of edible films. The solubility of proteins depends upon protein-solvent interactions having a lower free energy than the sum of the protein-protein and solvent-protein interactions (Mangino 1984). One of the most important physicochemical and functional properties of native whey proteins is their solubility over a wide range of protein concentrations, pH, temperature and ionic conditions (Dybing and Smith 1991; Morr and Ha 1993). Most of the hydrophobic and cysteine amino acid residues are located in the interior of the globular protein. Heating causes protein unfolding, exposure of the interior amino acids and increased protein-protein interactions, with resulting loss of solubility. At temperatures < 65 °C, the hydrophobic effect (major force favoring the folded state) dominates; whereas at higher temperatures, the configurational entropy (major force favoring the unfolded state) dominates (Albert 1989; Bryant and McClements 1998).

Figure 1 shows film protein solubility in water at 25 °C as a function of heat denaturation time and temperature. As heat denaturation time and temperature increased, film protein solubility in water decreased. Heat-denatured WPI film-forming solutions in a water bath at 70 °C gave the highest film protein solubility, even after 20 min. Denaturation in the water bath set at 90 °C and 100 °C for at least 10 min was required to achieve the lower solubilities, and further increase in denaturation time did not reduce film protein solubility. These values correspond, respectively, to the values found by Pérez-Gago and others (1999) for native films (that is, whey protein which has not undergone heat treatment) and heat denatured WPI films (that is, film-forming solution in water bath at 90 °C for 30 min).

Usually when describing film formation, the temperature that is recorded is the bath setting temperature. However, these temperatures do not usually correspond with the actual temperature of the film-forming solutions. At the water bath settings used in this experiment, the temperatures of the 200mL WPI solutions, recorded as soon as they were taken from the water bath, are reported in Table 1. Exposure to solution temperatures above 70 °C for at least 15 min is required to achieve insoluble films in water; and when temperature is increased, lower solution heating times are required to achieve similar film protein solubilities. These results agree with heat denaturation conditions required in gel formation. Typically in gel formation, whey protein solutions are held for between 5 to 60 min at temperatures between 70 and 90 °C, to ensure the correct degree of protein unfolding and aggregation (Barbut and Foegeding 1993; Bryant and McClements 1998). When comparing protein solubility values to the temperature of the solutions at each condition, we can draw a separation line for the time-temperature combination required to obtain water-insoluble films. These results clearly indicate the importance of controlling both variables, which will also be affected by the amount of the solution prepared, in order to achieve the desired solubilities.

Figure 1 also shows film protein solubility, when films were soaked in water at 100 °C for 4 min, as a function of film-forming solution heat-denaturation time and temperature. Again, increase in heat-denaturation time and temperature of the WPI solutions resulted in films with lower protein solubility. When comparing film protein solubility with films soaked in 25 °C water for 24 h, results are not significantly different (Figure 1). The disruption of the native 3-dimensional structure of the proteins as heat denaturation time and temperature increases, exposing SH groups formerly buried inside the molecules, enables the formation of covalent intermolecular disulfide bonds, which are accountable for film water-insolubility. Disulfide bonds are the strongest

Table 2—% Swelling of WPI films after soaking in water at 25 °C (24 h) and at 100 °C (4 min), for films formed from WPI solutions heated at selected temperatures and times

	Heat Denaturation Temperature (°C)[1]					
	80		90		100	
Time	Soaking		Soaking		Soaking	
(min)	25	100	25	100	25	100
5					184[e]	
10	247[c]		124[f]	360[a]	85[h]	226[d]
15	130[f]	371[a]	119[g]	247[c]	91[h]	180[e]
20	109[g]	348[b]	90[h]	256[c]	83[h]	182[e]

[1]Temperatures are heating-bath temperatures. Actual temperatures reached by film formulations are provided in Table 1.
% Swelling values are mean values of 3 replicates and 2 repeated observations per replicate.
Means with different superscripts are significantly different at $\alpha = 0.01$.

Table 3—Tensile Strength of WPI:Gly (70:30) films as a function of film-forming solution heat-denaturation time and temperature.

	Heat Denaturation Temperature (°C)[1]			
Time (min)	70	80	90	100
5	3.4 ± 0.4^{a}	3.8 ± 0.3^{a}	$8 \pm 2^{c,d}$	$8 \pm 3^{c,d}$
10	3.3 ± 0.1^{a}	7 ± 2^{c}	12 ± 2^{d}	10 ± 3^{d}
15	4.5 ± 0.2^{b}	12 ± 2^{d}	12 ± 2^{d}	12 ± 2^{d}
20	4.9 ± 0.2^{b}	14 ± 2^{d}	13 ± 2^{d}	$9 \pm 2^{c,d}$

Means with different superscripts are significantly different at $\alpha = 0.05$.
[1]Temperatures are heating-bath temperatures. Actual temperatures reached by film formulations are provided in Table 1.

Table 4—Young's Modulus of WPI:Gly (70:30) films as a function of film-forming solution heat-denaturation time and temperature.

	Heat Denaturation Temperature (°C)[1]			
Time (min)	70	80	90	100
5	156 ± 17^{a}	159 ± 24^{a}	$327 \pm 71^{c,d}$	342 ± 32^{c}
10	141 ± 10^{a}	299 ± 62^{c}	429 ± 59^{d}	419 ± 53^{d}
15	194 ± 12^{b}	$346 \pm 71^{c,d}$	427 ± 41^{d}	425 ± 28^{d}
20	192 ± 13^{b}	460 ± 42^{d}	472 ± 57^{d}	429 ± 28^{d}

Means with different superscripts are significantly different at $\alpha = 0.05$.
[1]Temperatures are heating-bath temperatures. Actual temperatures reached by film formulations are provided in Table I.

of the protein-protein interaction, and these bonds are not affected by changes in temperature (Bryant and McClements 1998). This could explain why film protein solubility does not increase as film soaking temperature is increased at 100 °C. When low temperature-short times are used to prepare WPI films, film formation relies mainly on hydrogen bonding and WPI film protein solubility in water is high, independent of film soaking temperature.

Film Solubility (Total Soluble Matter)

Consistent with the trend in percentages of protein solubility, increase in heat denaturation time and temperature gave less soluble films in water at both 25 °C and 100 °C. Film pieces formed using low denaturation temperatures and short times for film-forming solutions dissolved rapidly after being in contact with water. Hence, a total soluble matter of 100% was obtained (Figure 2). Whey protein film pieces formed from film forming solutions heated at high temperature and/or long time maintained their integrity throughout the whole film-soaking treatment. For these heat-denatured WPI films, glycerol represents most of the soluble matter, since glycerol is very hydrophilic. These results confirm those of Pérez-Gago and others (1999), where native WPI films were completely water soluble and heat-denatured WPI films were insoluble.

Results show a consistent trend in percentages of protein solubility and total soluble matter. Due to high energy intermolecular bondings, solubility of heat-denatured films is lower than the one of native whey protein films.

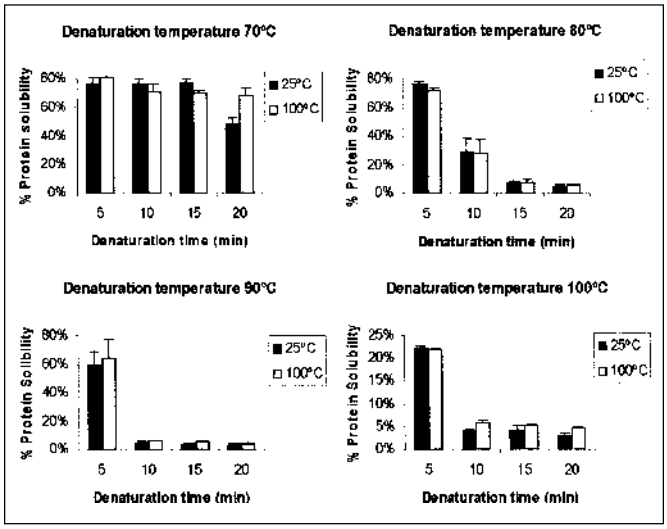

Figure 1—WPI film protein solubility in water at 25 °C (24 h) and 100 °C (4 min) as a function of film-forming solution heat-denaturation time and temperature. Temperatures on plot are heating-bath temperatures. Actual temperatures reached by film solutions are provided in Table 1.

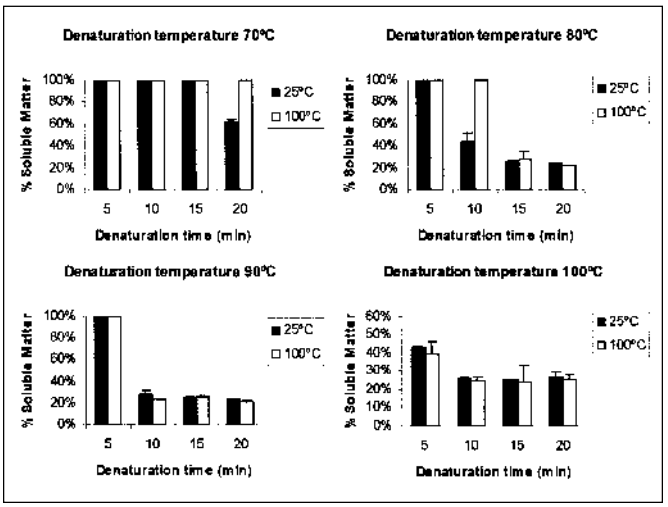

Figure 2—WPI film total soluble matter in water at 25 °C (24 h) and 100 °C (4 min) as a function of film-forming solution heat-denaturation time and temperature. Temperatures on plot are heating-bath temperatures. Actual temperatures reached by film solutions are provided in Table 1.

Table 5—Percent elongation at break of WPI:Gly (70:30) films as a function of film-forming solution heat-denaturation time and temperature.

	Heat Denaturation Temperature (°C)[1]			
Time (min)	70	80	90	100
5	7 ± 1^{a}	7 ± 1^{a}	3 ± 1^{d}	14 ± 3^{c}
10	$8 \pm 2^{a,b}$	18 ± 3^{c}	14 ± 3^{c}	14 ± 4^{c}
15	9 ± 0.4^{b}	17 ± 4^{c}	16 ± 3^{c}	15 ± 5^{c}
20	17 ± 5^{c}	18 ± 4^{c}	16 ± 5^{c}	18 ± 3^{c}

Means with different superscripts are significantly different at $\alpha = 0.05$.
[1]Temperatures are heating-bath temperatures. Actual temperatures reached by film formulations are provided in Table I.

Table 6—Oxygen permeability of heat-denatured and native WPI:Gly (70:30) films

Film Treatment	Test Conditions	Oxygen Permeability (cc μm/m² day kPa)
[c]Native	23 °C, 50% RH	78 ± 3^{a}
[c]Heat Denatured	23 °C, 50% RH	59 ± 3^{b}
[d]Heat Denatured	23 °C, 50% RH	66 ± 4
[d]Hydrolyzed Heat Denatured	23 °C, 50% RH	73 ± 3

Means with different superscripts are significantly different at $\alpha = 0.01$.
[c] OP values are mean and standard deviation of 3 replicates.
[d] Sothornvit and Krochta (2000)

Film Swelling

Table 2 presents the degree of swelling of the WPI films after soaking in water at 25 °C and at 100 °C, as film forming solution heat-denaturation time and temperature increased. Degree of swelling was only possible for those WPI films that kept their integrity when in contact with water. Consistent with soluble matter and soluble protein, the degree of swelling is an indication of the degree of protein cross-linking. As film solution heat denaturation temperature and time were increased, the degree of swelling decreased, indicating more extensive protein cross-linking. However, whereas water temperature did not affect protein solubility and total soluble matter of the WPI films, it was observed that WPI films soaked in 100 °C water were more swollen than when films soaked in 25 °C water, and this increase depended on the degree of denaturation. At similar film solution heat-denaturation times, WPI films from solutions heat-denatured at 80 °C experienced a higher degree of swelling in 100 °C water, compared to WPI films from solutions heat-denatured at 100 °C under the same solubility conditions. The higher swelling with 100 °C soaking might suggest that probably longer soaking times would increase solubility of the films by affecting hydration interactions, hydrogen bonding and hydrophobic interactions, since these decrease at high temperature (Bryant and McClements 1998).

Tensile Properties

Tables 3, 4 and 5 show the mechanical properties for WPI films from solutions denatured at different temperatures and times. As heat-denaturation time and temperature increased, tensile strength, Young's Modulus and percentage elongation increased. These results confirm those of Pérez-Gago and others (1999), that native films were less stiff, weaker and less extendible than heat-denatured films. The low energy bondings (mainly hydrogen bonding) and the globular structure of whey proteins in the native films could be accountable for these results. As globular whey protein unfolds, the covalent disulfide bonding of the heat-denatured whey protein films produces stronger films and enables the films to withstand higher deformations.

Oxygen Permeability

Table 5 shows OP of native WPI films (no heat treatment was used during film preparation) and WPI films prepared by heat denaturing the film forming solution in a water bath at 90 °C for 30 min. These results give information on film OP when the protein is in native and unfolded states. OP of native WPI films is significantly higher than OP of heat-denatured films, but of the same order of magnitude. These results contrast with Sothornvit and Krochta (2000), who concluded that type of protein structure (hydrolyzed compared to unhydrolyzed WPI films) did not affect film OP with the same plasticizer type and level. In spite of the different OP of native versus heat denatured WPI films, differences are small. The lower OP values for heat-denatured films may be related to their more linear (unfolded) structure, leading to higher cohesive energy density and lower free volume among polymer chains (Miller and Krochta 1997).

Conclusions

UNDERSTANDING THE STRUCTURE AND INTERACTIONS BEtween protein molecules in film formation are essential in order to form films with the desired solubility and mechanical properties. As heating time and temperature was increased, solubility of the films was decreased. No differences were observed in solubility between films soaked in 25 °C water for 24 h and films soaked in 100 °C water for 4 min. However, the degree of swelling was significantly larger for films soaked in 100 °C water, which indicates that perhaps longer soaking times at high water temperature could increase solubility of the films. Parallel to decrease film solubility, films became stiffer, stronger and more stretchable as heat denaturation time and temperature were increased. These results indicate that increase in covalent cross-linking as heat-denaturation of the whey protein increases is important to forming water-insoluble film and with higher tensile properties.

References

Albert T. 1989. Stabilization energies of protein conformation. In: Fasman GD, editor. *Prediction of protein structure and the principles of protein conformation*. New York: Plenum. p 161-192.

ASTM. 1995. Standard test method for oxygen gas transmission rate through plastic film and sheeting using a coulimetric sensor. Standard Designation: D3985-95. In *Annual Book of American Standard Testing Methods*, ASTM, Philadelphia, PA.

ASTM. 1997. Standard test method for tensile properties of thin plastic sheeting. Standard Designation: D882-97. In *Annual Book of American Standard Testing Methods*, ASTM, Philadelphia, PA.

Barbut S, Foegeding EA. 1993. Ca^{2+}-induced gelation of pre-heated whey protein isolate. J Food Sci. 58(4): 867-871.

Bryant CM, McClements DJ. 1998. Molecular bases of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. Trends Food Sci. & Tech. 9(4): 143-151.

Debeaufort F, Quezada-Gallo JA, Voilley A. 1998. Edible Films and Coatings: Tomorrow's Packaging : a Review. Critical Reviews in Food Science and Nutrition. 38(4): 299-313.

De Mulder-Johnston C. 1999. Thermal Analysis of, and Oil Migration through Films from Whey Protein Isolate. Ph.D. Dissertation, Univ. of California, Davis. p. 33-57.

Dybing ST, Smith DE. 1991. Relation of Chemistry and Processing Procedures to Whey Protein Functionality: A Review. Cultured Dairy Products J 26(1):4-12.

Gennadios A, Weller CL. 1991. Edible films and coatings from soymilk and soy protein. Cereal Foods World. 36(12): 1004-1009.

Guilbert S. 1986. Technology and application of edible protective films. In: Food packaging and preservation: Theory and practice. London: Elsevier Applied Science Publishing Co. p. 371-393.

Kester JJ, Fennema OR. 1986. Edible films and coatings: a review. Food Technol. 40(12):47-59.

Krochta JM, Baldwin EA, Nisperos-Carriedo M. 1994. *Edible coatings and films to improve food quality*. Lancaster: Technomic Publishing Company. 379p.

Krochta JM, De Mulder-Johnston C. 1997. Edible and biodegradable polymer

films: challenges and opportunities. Food Technol. 51(2): 61-72.
Mangino ME. 1984. Physicochemical aspects of whey protein functionality. J. Dairy Sci. 67(11): 2711-2722.
McHugh TH, Krochta JM. 1994a. Plasticized whey protein edible films: water vapor permeability properties. J. Agric. Food Chem. 59(2): 416-419.
McHugh TH, Krochta JM. 1994b. Water vapor permeability properties of edible whey protein-lipid emulsion films. JAOCS. 71(3): 307-312.
Miller KS, Krochta JM. 1997. Oxygen and aroma barrier properties of edible films: A Review. Trends in Food Science & Technol. 8(7): 228-237.
Morr CV, Ha EYW. 1993. "Whey Protein Concentrates and Isolates: Processing and Functional Properties," CRC Crit. Rev. Food Sci. Nutri., 33(6):431-476.
Pérez-Gago MB, Krochta, JM. 1999. Water vapor permeability of whey protein emulsion films as affected by pH. J. Food Sci. 64(4): 695-698.
Pérez-Gago MB, Nadaud P, Krochta JM. 1999. Water vapor permeability, solubility and tensile properties of heat-denatured versus native whey protein films. J. Food. Sci. 64(6): 1034-1037.
SAS Institute Inc. 1996. SAS/STAT® Users Guide, Version 6.12, 4th ed. Cary: SAS Institute, INC.
Shellhammer TH, Krochta JM. 1997. Whey protein emulsion film performance: effect of lipid type and amount. J. Food Sci. 62(2): 390-394.
Shimada K, Cheftel JC. 1989. Sulfhydryl group disulfide bond interchange during heat induced gelation of whey protein isolate. J. Agric. Food Chem. 37(1): 161-168.
Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150(1): 76-85.
Sothornvit R, Krochta JM. 2000. Oxygen permeability and mechanical properties of films from hydrolyzed whey protein. J. Agric. Food Chem. In Press.
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