

Relationship between the Microstructure and the Mechanical and Barrier Properties of Whey Protein Films

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This work was focused on the relationship between the microstructure and the mechanical and barrier properties of whey protein isolate (WPI) films. Sorbitol (S) and glycerol (G) were used as plasticizers and the pH was varied between 7 and 9. The films were cast from heated aqueous solutions and dried in a climate room at 23 °C and 50% relative humidity for 16 h. The microstructure of the films was found to be dependent on the concentration, the plasticizers, and the pH. When the concentration increased, a more aggregated structure was formed, with a denser protein network and larger pores. This resulted in increased water vapor permeability (WVP) and decreased oxygen permeability (OP). When G was used as a plasticizer instead of S, the microstructure was different, and the moisture content and WVP approximately doubled. When the pH increased from 7 to 9, a denser protein structure was formed, the strain at break increased, and the OP decreased.

Keywords: *Whey protein films; oxygen permeability; water vapor permeability; moisture content; critical gel concentration; sorbitol; glycerol*

INTRODUCTION

The microstructural characterization of biopolymeric films is an important element in understanding the film behavior and properties. Frinault et al. (1997) examined casein films with scanning electron microscopy (SEM) and transmission electron microscopy (TEM) and showed that the films had a more porous structure in the center than toward the edges. Banjeree and Chen (1995) used TEM to reveal the presence of residual milk fat embedded in the protein matrix of whey protein concentrate films. Atomic force microscopy was used by Lent et al. (1998) in a study of whey protein films topography. Lai and Padua (1997) used SEM to investigate zein films plasticized by oleic acid and showed that the oleic globules had separated from the zein matrix, which resulted in a more uneven top surface. Still, relatively few papers have used microstructural techniques to relate the microstructure to the film properties. The present study of the relationship between the microstructure and the mechanical and barrier properties of whey protein isolate (WPI) films has been focused on the effect of three different parameters: the concentration, the plasticizer, and the pH.

How changing the concentration of WPI could affect the mechanical properties was investigated in a previous paper (Anker et al., 1999). Varying the concentration over and under the critical gel concentration (c_g) elucidated the influence of the polymer network on the film properties. Strain at break (ϵ_b) showed a maximum at the c_g , thus implying that the most favorable structure regarding the ability of the films to stretch was formed at this concentration. A maximum was also observed for Young's modulus (E) and stress at break (σ_b). The reduced mechanical properties for films formed

below and above c_g indicated that different protein network structures were formed.

How varying the concentration of WPI could influence the barrier properties was studied by McHugh et al. (1994). The paper showed that 8% (w/w) WPI films had lower water vapor permeability (WVP) values than 12% (w/w) WPI films. The authors suggested that this might be due to an increased number of air bubbles in the 12% (w/w) WPI films. Although this showed how large an effect of the concentration has on the barrier properties, several other factors are known to affect the permeability: the microstructure, the plasticizer, the density, the orientation, cross-linking, and the molecular weight of the polymer chains, the nature of the permeant, etc. (Pascat, 1986; Miller and Krochta, 1997). So far no papers appear to have demonstrated the effect of an increased concentration on the microstructure of WPI films.

The plasticizers often used in WPI films are glycerol (G) and sorbitol (S). S was used as a plasticizer in our previous papers (Anker et al., 1998, 1999) due to its ability to bind less water than G and, thereby, provide a better barrier against water vapor at low and intermediate relative humidity (RH). This may be exemplified by McHugh et al. (1994), who showed that WPI/S = 1.7 films exhibited significantly lower WVP values than WPI/G = 1 films. Unfortunately, these plasticizers migrate during the lifetime of the WPI films. This phenomenon could be desirable if the WPI film is intended for use as a carrier of, for example, antioxidants and antimicrobial agents that will migrate into the packed food and prolong its shelf life. On the other hand, it could be undesirable since it affects the film properties and makes the films stiffer and less stretchable. This aging is, nevertheless, hard to avoid, since it is a natural process that occurs when low molecular substances are added to polymeric films.

The main difference between the two plasticizers in the effect of migration is that S crystallizes on the

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Table 1. Physical Properties of Glycerol and Sorbitol

	glycerol	sorbitol
formula	C ₃ H ₈ O ₃	C ₆ H ₁₄ O ₆
molecular mass (Da)	92	182
form	liquid	powder
melting point (°C)	18 ^{a,c}	98–100 ^{b,c}
boiling point (°C)	290 ^{a,c}	295 ^{d,e}

^a From Nordling and Österman (1987). ^b From Sigma-Aldrich (1997). ^c Pressure 100 kPa. ^d From Weast et al. (1972). ^e Pressure 0.5 kPa.

surface of the WPI films, which gives the films a slightly undesirable appearance, whereas the migration of G is not that easily detectable by optical examination. This is because S is crystalline at room temperature, whereas G is a clear liquid. The time scale for the first signs of crystallization of S on the film surface can be rather long (up to four months) and depends on the amounts of plasticizer used. The more plasticizer used, the faster the detection. However, if the films are to be used on a short time scale, or used inside foods, the consumer will not notice this crystallization. It is nevertheless important to know the effect of this migration, and thus, the aging of WPI films plasticized with glycerol and sorbitol will be investigated in a separate study. Park et al. (1994) studied wheat gluten and corn zein films and showed that when the G migrated during the storage time of 20 days, both the tensile strength and elongation decreased. The authors implied that even when the G is well-dispersed in the protein matrix the plasticizer migrates because of the binding limitations between the protein molecules and G. The migration rate was also suggested to be dependent on the types of functional groups, polarity, and structure of the film matrix.

There were certain reasons for using G parallel with S in the present investigation: the crystallization of S on the film surface and the different physical properties of the plasticizers (Table 1), which imparts different properties to the WPI films. Several papers have been published where S and G have been used as plasticizers with biopolymeric films. Fairley et al. (1996) showed that WPI films that contained sodium dodecyl sulfate and were plasticized with S were more extensible than films plasticized with G. McHugh and Krochta (1994) studied WPI/S = 1 and WPI/G = 2.3 films and presented similar tensile strength values. They concluded that a higher amount of S than G was needed to obtain similar tensile properties and suggested that the smaller size of the G molecule enables it to influence the film properties more readily than the S molecule. However, no papers have focused on the microstructure of WPI films plasticized with S and G. In the present study the ratio of WPI/S = 1.2 and WPI/G = 2.1 was determined so films with similar mechanical properties would be investigated. The WPI/plasticizer ratio was held constant to eliminate the plasticizing effect.

The effect of changing the pH value between 7 and 9 has previously been investigated (Anker et al., 1998, 1999). The results showed that the WPI films had a higher ϵ_b when the pH increased. This result is in agreement with previous findings for casein films, in which the ϵ_b increased when the pH increased from 7 to 9 (Frinault et al., 1997). Similar results have also been presented for peanut protein films (Jangchud and Chinnan, 1999) and soy protein isolate films (Gennadios et al., 1993).

The aim of this paper was to study the relationship between the microstructure and the mechanical and

barrier properties of WPI films. The focus has been to study the effect of the concentration, the plasticizer, and the pH. The concentration of WPI was varied below, at, and above the c_g to elucidate the influence of the varied concentration. S and G were used as plasticizers, and the pH was varied between 7 and 9 to investigate if different microstructures were formed.

MATERIALS AND METHODS

Materials. WPI was obtained from MD Foods Ingredients (Videbaek, Denmark). WPI (Lacprodan PSDI-9224) is a functional WPI used for protein fortification of clinical nutrition products as well as sports foods. The WPI powder had a dry content of $93 \pm 2\%$ protein ($N \times 6.38$), 0.2% fat, 0.2% lactose, 5.5% moisture, and 4.0% minerals and a pH between 6.5 and 7.0 (0.10% solution). The concentration of proteins in the WPI powder was 74% β -lactoglobulin, and 18% α -lactalbumin, 6% bovine serum albumin, and 2% immunoglobulins. For further details regarding the molecular mass and cysteine groups of each protein, see the work of Anker et al. (1999). Glycerol (G) and sorbitol (S) (>98% purity) were used as plasticizers and were obtained from BDH Laboratory Supplies (Poole, England) and Sigma Chemical Co. (St. Louis, MO), respectively. G and S are colorless, odorless, and sweet-tasting alcohols. Their physical properties are listed in Table 1. G is obtained from oils and fats as byproduct in the manufacture of soaps and fatty acids and is commonly used in cosmetics and confectioneries as a solvent, plasticizer, and sweetener. S occurs widely in nature in most fruits, such as black grapes, apples, plums, and cherries, but is produced commercially by hydrogenation of glucose. S is utilized in processed foods, confectioneries, toothpaste, and other personal care products as a stabilizer, plasticizer, emulsifier, and bodying agent (Budavari et al., 1989).

Dynamic Oscillatory Measurements. Dynamic oscillatory measurements were performed in a Bohlin VOR Rheometer (Bohlin Rheology, Chichester, U.K.) to find the critical gel concentration (c_g) for the WPI/G = 2.1 film-forming solutions at pH 7, 8, and 9. The c_g is the minimum concentration needed to form a gel under the actual gelation process conditions. A 10 mL solution was poured in a double gap (DG) measuring system and paraffin oil was put atop the solution to avoid moisture loss. The DG outer cylinder diameters were 21.8 and 27.5 mm and the inner cylinder diameters were 24 and 25 mm. The frequency was set to 1 Hz and the strain at 1.6×10^{-3} , which is within the linear region for the gels formed.

The temperature in the rheometer was varied to simulate the heating, casting, and cooling of the film-formation procedure. The solutions were heated in the rheometer at a constant heating rate of 6 °C/min to 76.5, 70.5, and 68 °C for pH 7, 8, and 9, respectively. This resulted in an average heating time of 11–13 min. Hence, the solutions were heated to 10 °C above the denaturation temperature of β -lactoglobulin, the dominating whey protein. The denaturation temperatures were determined by differential scanning calorimetry to be 66.5, 60.5, and 58 °C for pH 7, 8, and 9, respectively, at a heating rate of 10 °C/min (Hegg, 1980). Different maximum temperatures for each pH were used, because the denaturation temperature, and thus the gelling, varies with pH. Thereafter the solutions were allowed to cool to 20 °C, with the steepest cooling rate in the beginning. Hence, the cooling rates were subsequently –6, –3, and –1 °C/min. This resulted in a cooling time of ≈ 20 min.

The network formation was monitored in the rheometer by studying the elastic part, the storage modulus (G'), and the viscous part, the loss modulus (G''), of the complex shear modulus (G^*) as a function of time. G^* is related to the storage and loss modulus through the relation $G^* = G' + jG''$, where $j^2 = -1$. The phase angle (δ) indicates the degree of viscosity to elasticity and was calculated as $\tan \delta = G''/G'$. The gel point was determined as the instant when $G' = G''$, that is, when $\delta = 45^\circ$. A mechanical spectrum from 0.01 to 1 Hz was recorded at 20 °C at the end of the temperature curve. The δ at 1 Hz

Table 2. Experimental Design

		pH 7 ^a	pH 8 ^a	pH 9 ^a
WPI/S = 1.2	low concentrated	7.7	8.1	7.3
	c_g	11.7	12.1	11.3
	high concentrated	15.7	16.1	14.3
WPI/G = 2.1	c_g	10.8	11.7	11.0

^a % (w/w) WPI.

was recorded for each concentration tested. Linear regression of the δ vs the concentration was used to estimate the critical gel concentration (c_g) as the concentration when the $\delta = 45^\circ$.

Experimental Design. The experimental design in Table 2 was chosen to be able to study the microstructure and the mechanical and barrier properties below, at, and above the c_g , as well as the effect of the two different plasticizers used. The concentration of WPI was varied 4% (w/w) over and under the c_g at each pH value, except at pH 9. At 4% (w/w) WPI over c_g at pH 9, it was very difficult, almost impossible, to cast films due to the enhanced aggregation, and the subsequent gelation, that occurs at high concentrations. Therefore, the highest concentration of WPI used was 14.3% (w/w) at pH 9, which was 3% (w/w) WPI above the c_g .

Film Formation. The concentration of WPI was varied according to the experimental design displayed in Table 2. The ratios of WPI/S = 1.2 and WPI/G = 2.1 were held constant. The concentration of WPI and S was based on the dry weight. The solutions were mixed, adjusted for pH, degassed, and heated to 76.5, 70.5, and 68 °C for pH 7, 8, and 9, respectively, at a heating rate of $\approx 6^\circ\text{C}/\text{min}$. The cast solutions were allowed to cool and dry at room temperature for ≈ 4 h and were then dried in a climate room at 23 °C and 50% RH for 16 h. To ensure that the films could be peeled intact from the casting surface, the Petri dish was covered with a hydrophobic surface of Teflon FEP film (Norton Performance Plastics Corp., Akron, OH). A 15.4 g solution was applied to each Petri dish to minimize thickness variations. Films were preconditioned in a climate room at 23 °C and 50% RH, for at least 48 h prior to all testing. For further details regarding the film formation, see Anker et al. (1998).

Transmission Electron Microscopy. Small film strips, ≈ 5 mm long and 3 mm wide, were cut from the ready films, and small triangular incisions were made at the end of each filmstrip to be able to distinguish between the top (dried in contact with air) and the bottom (dried in contact with Teflon) of the films. Filmstrips were fixed for 2 h in 37% formaldehyde fumes and thereafter postfixed for 2 h in 2% osmium tetroxide fumes. The samples were then dehydrated for 15 min each in a graded ethanol series: 50%, 75%, and three times at 99.5%. Treated filmstrips were then embedded in increasing concentrations of acrylic resin (LR White Resin Medium Grade, Standard Supplies AB, Källered, Sweden): 2 h in 50%, overnight in 75%, ≈ 8 h in 100%, overnight in 100%, and ≈ 4 h in 100%. Subsequently, the polymerization of the resin proceeded at 60 °C for 20 h. Ultrathin sections, ~ 90 nm, were cut on a diamond-knife using an Ultracut E ultramicrotome (Reichert Jung). Sections were picked up on copper grids and stained with 5% uranyl acetate (1 h) and 0.3% lead citrate (1 min). The microstructure of the film cross sections was observed at 100 kV ($\times 3597$) using a LEO 906E transmission electron microscope (LEO Electron Microscopy Ltd., Cambridge, England) with a high-performance, low-intensity charged coupled device (CCD) camera. The preparations of each experimental point were duplicated for the WPI/S = 1.2 films to ensure that the technique resulted in accurate micrographs. The preparations of each experimental point for the WPI/G = 2.1 films were reproduced once.

Film Thickness. Before testing, the thickness of the films was measured by a digital micrometer (model IDC-112CB, Mitutoya Corp., Tokyo, Japan) at five positions along the rectangular strips for the mechanical properties, and at the center and at four positions round the perimeter for the OP and WVP. The mechanical properties, OP, and WVP were calculated using the average thickness for each film replicate.

Mechanical Properties. A texture analyzer (model TA-XT2, Stable Micro Systems, Godalming, England) was used to determine mechanical properties at large deformations in accordance with ASTM D882-91. The films were tested in a climate room at 23 °C and 50% RH. Initial grip separation and crosshead speed were set to 50 mm and 24 mm/min, respectively. The tested filmstrips were 80 mm long and 6 mm wide. Force and elongation were recorded during extension, and Young's modulus (E), stress at maximum force (σ_y), stress at break (σ_b), and strain at break (ϵ_b) were calculated. These properties derived from the tensile test are often also referred to in the literature as modulus of elasticity, yield strength, tensile strength, and elongation. Yield strength is the tensile stress at which the first sign of nonelastic deformation occurs.

Moisture Content. MC was determined by drying in an oven (model T6060, Heraeus, Mölndal, Sweden) at 105 °C for 24 h. Small test specimens were cut and put on glass Petri dishes, and the weights before and after the oven drying were recorded. MC was calculated as the percentage of weight loss based on the original weight, in accordance with ASTM D644-94. At least 15 replicates of each experimental point were evaluated.

Water Vapor Permeability. WVP was determined according to ASTM E96-90 and corrected for the stagnant air gap inside test cups for hydrophilic films using the WVP correction method (McHugh et al., 1993). A climate room containing fans was used to test the WVP, and the fan speed was set to achieve an air velocity of >152 m/min above the test cups to avoid stagnant air and to minimize mass transfer resistance above the films. The climate room was set at 23 °C and 50% RH. Distilled deionized water was placed in the bottom of the test cups to expose the film to a high RH inside the test cups. A fixed amount of water was used so that the stagnant air gap height inside the cups was less than 14 mm to ensure accurate determination of the WVP. The surface of the films that had been dried in contact with the hydrophobic Teflon film was facing the high RH. After the films were mounted, the whole assembly was weighed. Weighing (with an accuracy of 0.01 g) was repeated at 30 min intervals. Steady state was achieved in <2 h, and thereafter the weight of the assembly was recorded every 2 h during the first 8 h, and finally after 24 h. At least nine replicates of each experimental point were evaluated. In all cases, a linear regression of the steady-state data yielded $R^2 > 0.999$. Due to the stagnant air gap inside the cups, the RH on the underside of the films varied between 72 and 80%. Consequently, the gradient varied between 50–72% and 50–80%. For further details regarding the cups and the WVP measurements, see the work of Anker et al. (1998).

Oxygen Permeability. OP was determined in accordance with ASTM F1307-90 using a MOCON unit (OXTRAN 2/90, Modern Control, Inc., Minneapolis, MN). Film samples were double-masked with aluminum foil masks supplied by the manufacturer with an effective film test area of 5 cm². The surface of the films that had been dried in contact with the hydrophobic Teflon film was facing the 100% oxygen. Testing was performed at 23 °C and 50% RH.

RESULTS AND DISCUSSION

The results from varying the concentration, the two plasticizers used, and the different pH values applied will be presented side by side in this study. First, the critical gel concentration (c_g) of the film-formation solutions is presented. The c_g is a very important parameter, since the film properties are highly affected when the concentration of WPI varies around this concentration. Second, the microstructure of the films is shown. The microstructural characterization is essential when a complete understanding of the film behavior is required, and therefore the microstructure is related to all of the studied film properties. Third, the mechanical properties are presented. The effect of

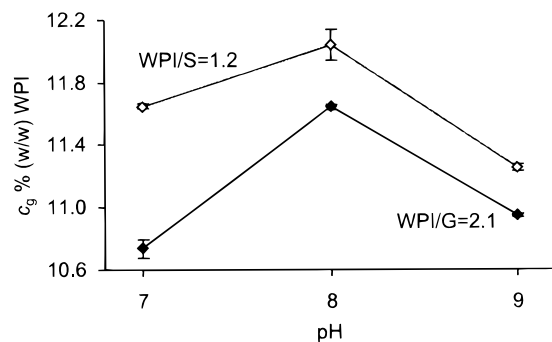


Figure 1. c_g of the WPI/S = 1.2 and WPI/G = 2.1 film-forming solutions as a function of pH. Error bars show the \pm 95% confidence interval.

changing the concentration below and above the c_g will be elucidated. Finally, the barrier properties of the films are shown. The effect on the OP and WVP of changing the concentration below and above the c_g will be investigated, as well as the effect of the MC on the WVP.

Since one objective of this study was to compare the effect of two plasticizers, data have been extracted from our previous study (Anker et al., 1999) where only S was used. Consequently, data regarding the c_g , the mechanical properties, and the MC have been incorporated in Figures 1, 5, 6, and 8, respectively.

Critical Gel Concentration (c_g). *Effect of the Plasticizers.* When the relative concentration of plasticizer decreases, the c_g decreases. In Figure 1, the c_g for the WPI/S = 1.2 and WPI/G = 2.1 film-forming solutions is shown. The c_g for the WPI/G = 2.1 solutions is lower than for the WPI/S = 1.2 solutions. This is probably due to the decreased relative concentration of plasticizer. Hence, fewer plasticizer–protein interactions occur, which enhances the protein aggregation and gelation and, consequently, lowers the c_g . Anker et al. (1999) showed that almost the same c_g values were achieved for the WPI/S = 1.2 solutions when a slower cooling rate was applied as for the c_g values presented for the WPI/G = 2.1 solutions in Figure 1. We explained this by the exposure of the whey protein molecules to higher temperatures for a longer time, which enhances the protein aggregation and gelation. Thus, the c_g decreases.

Determining the exact point at which a material passes from bulk behavior, from that of a liquid to that of a gel is not a simple procedure (Power et al., 1998). The dynamic oscillatory measurement method used both in this present work and the preceding one (Anker et al., 1999), has proven to be reliable for determining the c_g . Extensive measurements are, however, necessary, since the concentration needs to be varied in small steps around the final c_g .

Effect of the pH. The two plasticizers show a maximum at pH 8 when the pH is varied. The explanation of this behavior with pH is the proposed structural changes occurring in the gel network when the pH is increased. These changes will be thoroughly discussed under the “microstructure”.

Microstructure. The microstructural characterization of the WPI films in this study has been extensive, and many micrographs have been taken to make sure that the micrographs presented in this study are representative of each experimental point examined. The reason for the extensive measurements was to eliminate the experimental error which might occur when a very small part of the films is analyzed during

the microstructural characterization, compared to the much larger samples used when the mechanical and barrier properties are measured.

Effect of the Drying Rate and the Film Thickness. Different structures are formed at the top and bottom, compared to the center, of each film. This is illustrated in Figure 2, with an 11.3% (w/w) WPI/S = 1.2 film at pH 9, where the micrographs are taken from the same film replicate. A more porous structure is formed in the center, which probably is due to a slower mass transfer of water and, consequently, a slower drying rate. The protein matrix has time to form a more aggregated structure, which leads to the formation of larger pores. Water evaporates at the top, and the faster drying rate results in a denser structure with smaller pores. A similar structure is formed at the bottom. Here, the mass transfer of water may be less important, but the pressure of the total volume results in a less porous structure. This phenomenon was observed for all experimental points examined, but was less pronounced at low concentrations due to the less aggregated structures formed at these concentrations (Figure 3a–c). Thus, the microstructure was observed in the center of each film in Figures 3 and 4 to ensure representative micrographs. Several other researchers have shown that the microstructure in the center is different from the top and bottom of biopolymeric films. Frinault et al. (1997) examined casein films that had been formed by a wet-spinning method and had a more porous structure in the center than toward the edges. The authors suggested that, after the crust had been formed, water exclusion in the center took place, which might explain the less dense matrix in the center of the film. Similar results were reported by Gallant et al. (1984), for spun pea and fababean proteins.

Effect of the Drying Surface. Different surfaces are formed if the films have been dried in contact with air or in contact with the Teflon-covered Petri dish. At the top (Figure 2a) a slightly uneven surface is seen, compared to the smooth surface formed at the bottom (Figures 2c). The smooth surface is due to the casting of the film-formation solutions on the flat Petri dish covered with the Teflon film. The slightly uneven surface is, however, difficult to avoid, since the solutions are dried in contact with air. Therefore, all of the WVP and OP measurements were made with the smooth (bottom) surface facing the high RH and 100% oxygen to ensure accurate test results. Furthermore, the outer layer of the top and at the bottom shows a denser structure. This indicates that thin crusts have been formed at the surfaces, that at the top being more pronounced. Frinault et al. (1997) also showed a denser structure at the periphery of casein films. It could also be seen that the films were slightly uneven at the top, as shown in this present work. Lai and Padua (1997) reported similar results for zein films plasticized with oleic acid. The bottom of the films was featureless, compared to the rougher surface at the top. According to the authors, the latter effect was due to fat globules from oleic acid that had separated from the zein matrix when the solvent had evaporated. The latter result is interesting since producing emulsion films by mixing lipids with a protein or polysaccharide matrix, where the lipid migrates toward the surface during the drying process, could improve the barrier against water vapor. The use of this technique with our WPI films is investigated in a separate study.

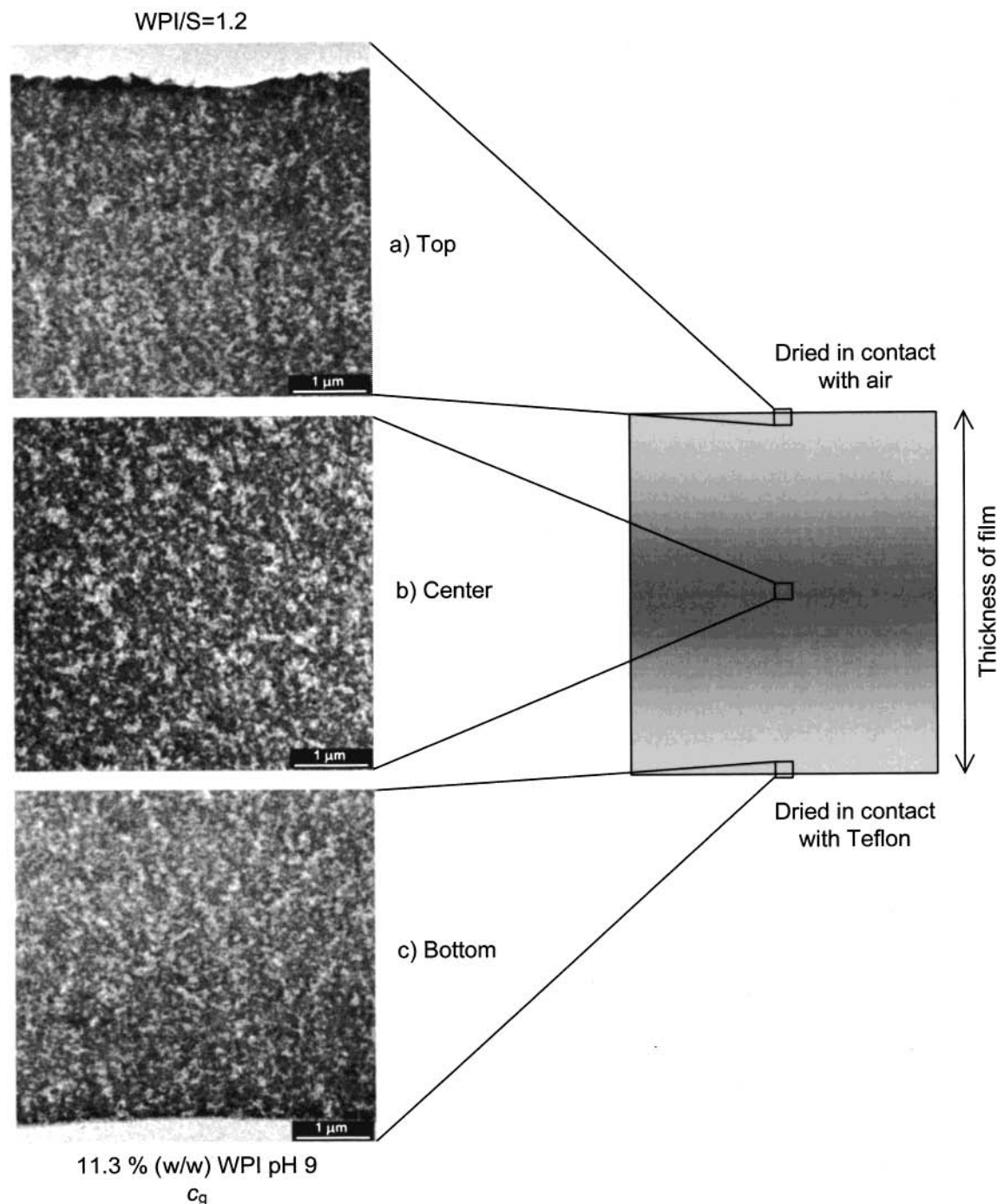


Figure 2. Representative transmission electron micrographs of a WPI/S = 1.2 film at the top, center, and bottom ($\times 3597$). The experimental point represented by the c_g at pH 9 (11.3% (w/w) WPI). Dark areas represent the protein matrix and white areas the water and plasticizer phase.

Effect of the Concentration. When the concentration increases, more aggregated structures are formed. This is shown in Figure 3, where the microstructure of WPI/S = 1.2 films at pH 7, 8, and 9, is presented. Below c_g , a fine WPI structure is formed (Figure 3a–c), at c_g a more aggregated structure is formed (Figure 3d–f), whereas at high concentrations a highly aggregated structure with larger pores is formed (Figure 3g–i). This change in the microstructure is strongly correlated with the mechanical and barrier properties that will later be presented.

Effect of the Plasticizers. The overall protein structure is different when S is used as plasticizer instead of G, and the differences can be observed by comparing

Figures 3d–f with 4a–c. The pores tend to be more and smaller when S is used, compared to the fewer and larger pores seen when G is used. There is also a difference in the pore size when the pH changes from 7 to 9 for the WPI/S = 1.2 films, which cannot be found for the WPI/G = 2.1 films. At pH 7 (Figure 3d,g), the pores are smaller and more homogeneous compared to the pores seen at pH 9 (Figure 3f,i).

Effect of the pH. When the pH increases, a denser protein structure is formed. This can be seen for the WPI/S = 1.2 films as more pronounced darker areas (protein phase) at pH 9 (Figure 3c,f,i) compared to the structures formed at pH 7 (Figures 3a,d,g). The same may be observed for the WPI/G = 2.1 films in Figure 4.

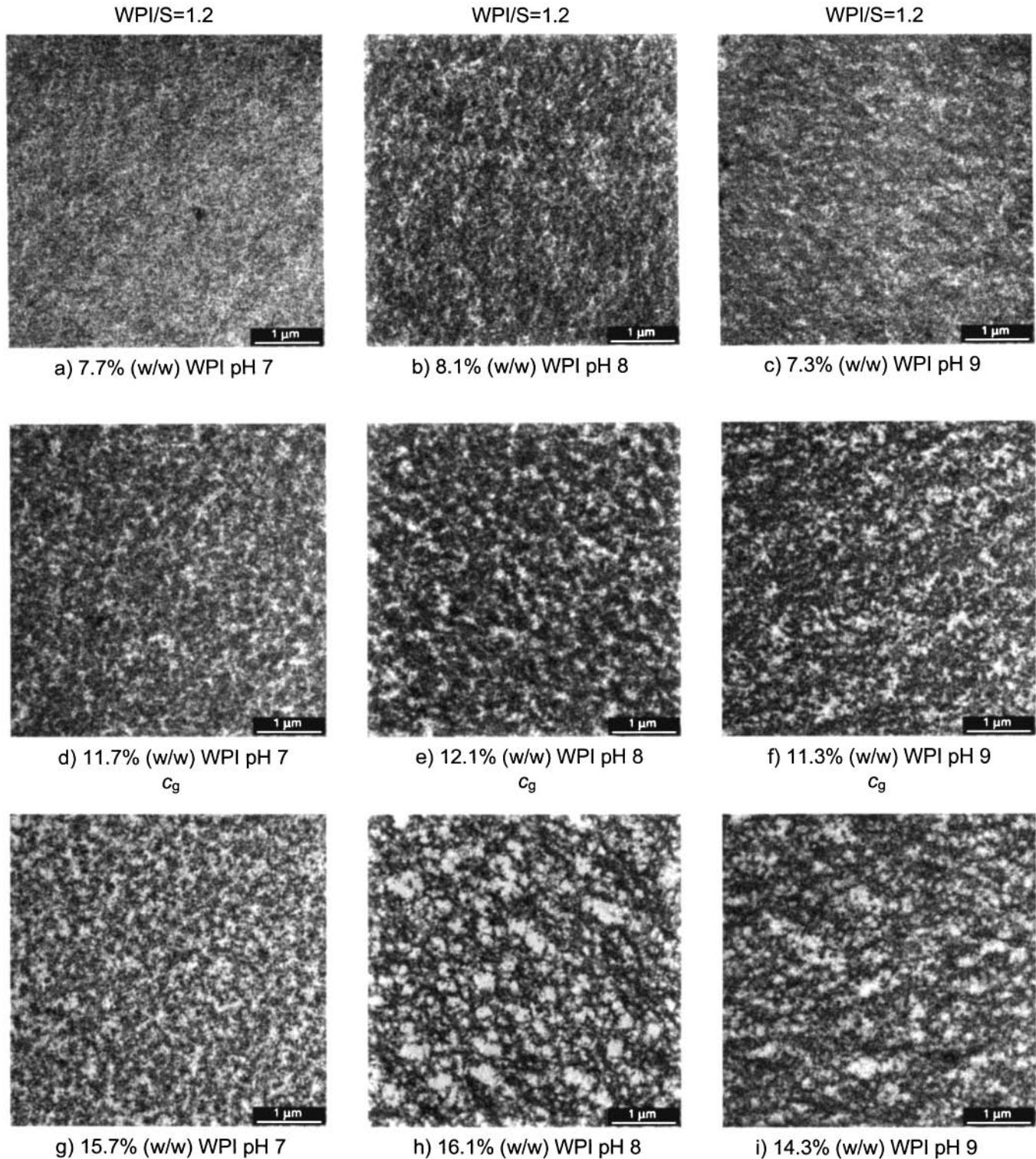


Figure 3. Representative transmission electron micrographs of WPI/S = 1.2 films at different concentrations and pH ($\times 3597$). Dark areas represent the protein matrix and white areas the water and plasticizer phase. Micrographs are taken from the center of each film replicate.

Here the protein matrix is obviously darker at pH 9 (Figure 4c) compared with pH 7 (Figure 4a). The microstructure of the WPI films in Figures 3 and 4 shows that structural differences between pH 7 and 9 can be distinguished, and that films made at pH 8 have some kind of intermediate structure. This verifies the observations with regard to pH that have been made in our previous studies (Anker et al., 1998, 1999). Frinault et al. (1997) reported that casein films produced at pH 9 had a denser structure than film formed

at pH 7. They used a wet-spinning process to form films and suggested that a high apparent viscosity was consistent with the formation of denser films.

Few papers have been published on the effect of alkaline pH on whey protein films, and very few, if any, have reported structural differences between pH 7 and 9. Fortunately, the behavior of whey protein films is very similar to whey protein gels, which means that the results are comparable. This is due to the dominating protein in whey, β -lactoglobulin (β -Lg), which has been

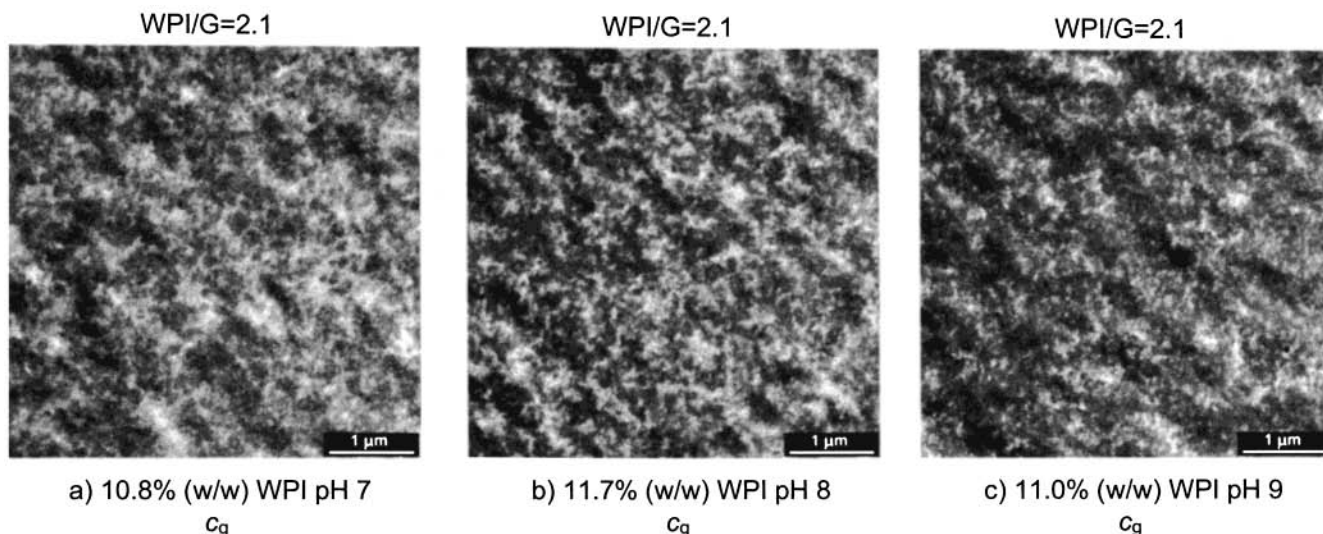


Figure 4. Representative transmission electron micrographs of WPI/G = 2.1 films at different concentrations and pH ($\times 3597$). Dark areas represent the protein matrix and white areas the water and plasticizer phase. Micrographs are taken from the center of each film replicate.

shown to influence the behavior of whey protein films (Anker et al., 1998) and gels (Hermansson, 1979, 1986; Stading and Hermansson, 1990, 1991; Langton and Hermansson, 1992; Stading et al., 1992, 1993). The mechanisms that may explain the denser network structure formed at pH 9 for the WPI films are discussed below.

Boye et al. (1997) reported that β -Lg gels at pH 8.6 had a denser protein structure compared to that at pH 7. According to the authors, the denser protein structure might be due to an enhanced gel formation at alkaline pH values. The enhanced gelation was reported to be partly due to the unfolding of the β -Lg molecule and partly due to the increased thiol/disulfide (SH/S-S) interchange reactions. The unfolding is initiated by the increased intramolecular repulsive forces at alkaline pH (Casal, 1988) and is similar to mild heat treatment. The unfolding is also indicative of the reduced thermostability at alkaline pH values, which results in a decreased denaturation temperature of β -Lg (Hegg, 1980). A partially unfolded protein requires less heat to denature than native proteins (Ma and Harwalkar, 1988; Waissbluth and Grieger, 1974). In the present study, different heating temperatures for each pH value were used, because the denaturation temperature, and thus the gelling, varies with pH. Nevertheless, at pH 9, enhanced gelation at high concentrations can be observed, which may be indicative of an increased gelation at alkaline pH values, similar to what was reported by Boye et al. (1997). The importance of the SH/S-S interchange reactions, however, has been widely discussed in the past and will most certainly be further discussed in the future. It is believed that, when the pH is increased above ~ 8 , SH/S-S interchange reactions or thiol/thiol (SH/SH) oxidation reactions can occur upon heating, and intermolecular disulfide (S-S) bonds can be formed (DeWit and Klarenbeek, 1983; Li-Chan, 1983; Mangino et al., 1987; Kinsella and Whitehead, 1989; Mleko and Foegeding, 1999).

Microstructure in Relation to the Mechanical Properties. *Effect of the Concentration.* When the concentration increases, the strain at break (ϵ_b) shows a maximum at the c_g for all pH values (Figure 5). This implies that the aggregated structures formed at this concentration (Figure 3d-f) are the ones that are most

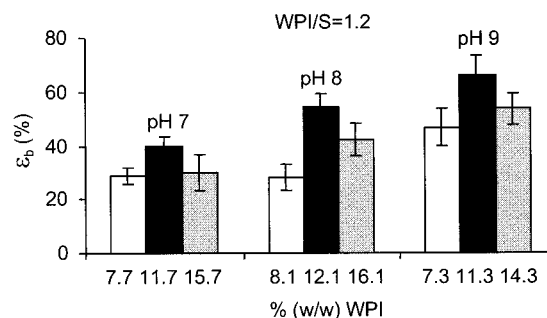


Figure 5. Strain at break (ϵ_b) of WPI/S = 1.2 films as a function of WPI concentration and pH. Error bars show the $\pm 95\%$ confidence interval.

conductive to stretch. The highly aggregated structures above c_g (Figure 3g-i) are formed due to the enhanced and fast gelling that occurs at these concentrations. Below c_g , the protein network is mainly formed during the drying process and results in fine protein structures (Figure 3a-c). When water evaporates, the concentration increases, and a sustainable protein matrix can be formed. The films are weaker and less extensible, but still show acceptable mechanical properties. In our previous paper a maximum was also observed for Young's modulus (E) and stress at break (σ_b) when the concentration varied above and below the c_g (Anker et al., 1999).

Effect of the Plasticizers. The stress and strain curves from the tensile test are displayed in Figure 6. The ϵ_b is higher for the WPI/S = 1.2 films compared with the WPI/G = 2.1 films (Figure 6). This is probably due to the increased amount of S used as a plasticizer. Fairley et al. (1996) obtained similar results with WPI films that were plasticized with S and contained sodium dodecyl sulfate, and were more extensible than films plasticized with G.

The other mechanical properties in Figure 6 are in the same range, probably due to the greater plasticizing effect of G. Several researchers have shown the greater plasticizing effect of glycerol compared to S. McHugh and Krochta (1994) studied WPI/S = 1 and WPI/G = 2.3 films and presented similar tensile strength values. They concluded that a higher amount of S than G was

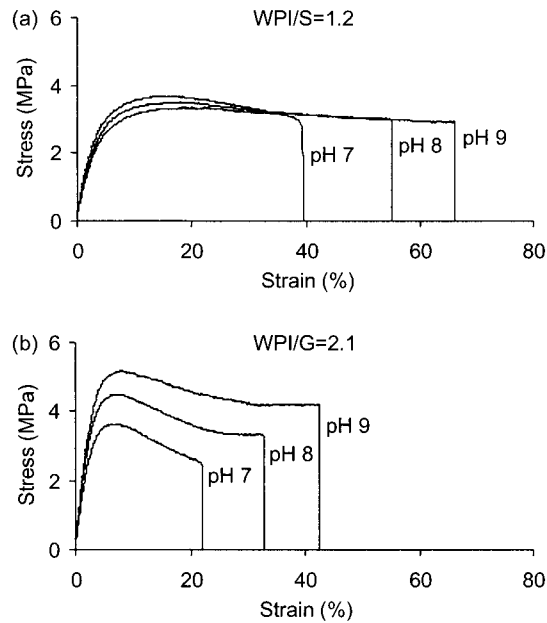


Figure 6. Representative stress and strain curves of (a) WPI/S = 1.2 and (b) WPI/G = 2.1 films. Each experimental point is represented by its c_g .

needed to obtain similar tensile properties and suggested that the smaller size of the G molecule enables it to influence the film properties more readily than the S molecule. Similar results were presented by Fasold et al. (1995), who showed that the molecular size of the plasticizer is of importance, since the glass transition temperature (T_g) decreased more with G used as plasticizer instead of S. Cuq et al. (1997) reported that G seemed to have a slightly higher plasticizing effect than S when large amounts of plasticizer were used with myofibrillar protein-based films.

The WPI/G = 2.1 films show an increased E , stress at maximum force (σ_y), and σ_b when the pH increases (Figure 6b). This effect cannot be seen with the WPI/S = 1.2 films (Figure 6a), but the effect might be hidden due to the increased, by weight, amount of S used as a plasticizer. The difference in behavior may also be due to the different physical features of the plasticizers, which can impart different properties to the ready films.

Effect of the pH. When the pH increases, the ϵ_b increases (Figures 5 and 6). In Figure 6, the representative stress and strain curves of WPI/S = 1.2 and WPI/G = 2.1 films are shown. The curves clearly show an increased ϵ_b when pH increases from 7 to 9, for both plasticizers used. This can also be seen in Figure 5 for the WPI/S = 1.2 films at different concentrations. Similar results with regard to pH have been seen in our two previous investigations (Anker et al. 1998; 1999) and are in agreement with previous findings with casein films, in which the ϵ_b increased when the pH increased from 7 to 9 (Frinault et al., 1997). Jangchud and Chinnan (1999) presented similar results for peanut protein films and Gennadios et al. (1993) for soy protein films. The other mechanical properties, such as the E , σ_y , and σ_b , also increased for the WPI/G = 2.1 films when the pH increased (Figure 6b). Jangchud and Chinnan (1999) showed that peanut protein films plasticized with G also increased their tensile strength when the pH increased.

Microstructure in Relation to the Barrier Properties. *Effect of the Concentration.* When the concentra-

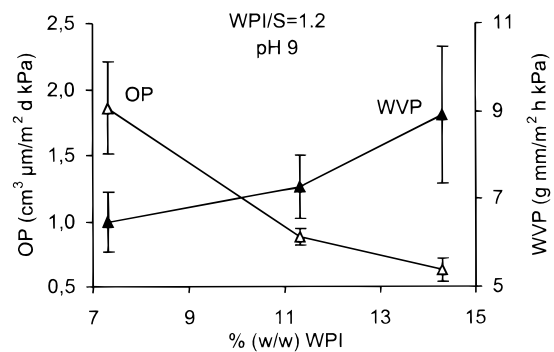


Figure 7. OP and WVP of WPI/S = 1.2 films at pH 9 as a function of WPI concentration. Error bars show \pm standard deviation for the OP values and the $\pm 95\%$ confidence interval for the WVP values.

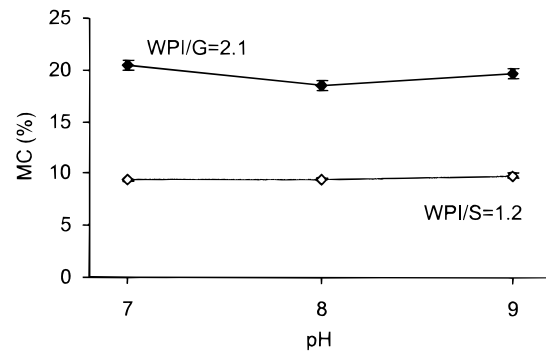


Figure 8. MC of WPI/S = 1.2 and WPI/G = 2.1 films as a function of pH. Each experimental point represented by its c_g . Error bars show the $\pm 95\%$ confidence interval.

tion increases, the WVP increases and the OP decreases (Figure 7). The effect of the concentration is represented by a WPI/S = 1.2 film at pH 9. The reason for the increased WVP is probably the larger pores formed at high concentration (Figure 3i), compared to the smaller pores formed at low concentration (Figure 3c). When the pore size increases, the water and plasticizer phase increases and, since the water molecules are hydrophilic, their easiest way through the film matrix is through the hydrophilic water and plasticizer phase. This phase increases as larger pores are formed, so that the WVP increases. Similar results were reported by McHugh et al. (1994), who showed that when the concentration increased from 8 to 12% (w/w) WPI, the WVP increased for the WPI films. One could also speculate that the easiest way for the hydrophobic oxygen molecules might be through the hydrophobic protein phase. At a high concentration, a denser protein structure is formed and, consequently, the OP is lowered due to the increased obstacle to the oxygen molecules passing through the more closely packed protein network. This is in agreement with Pascat (1986), who stated that a higher density decreases the permeability. Miller and Krochta (1997) further confirmed that the permeability is highly affected by how closely packed the polymer chains are.

Effect of the Plasticizers. Both the MC and WVP increased when G was used as a plasticizer instead of S (Figures 8 and 9). A clear distinction is that the MC and WVP are higher for the WPI/G = 2.1 films at each pH value. The increased MC is probably due to the more hygroscopic character of G, which therefore has a higher affinity than S to bind water at equivalent RH (Sicard and Leroy, 1983; Van Soest and Knorren, 1997). The

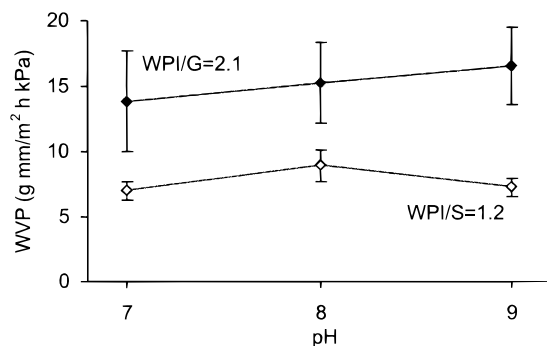


Figure 9. WVP of WPI/S = 1.2 and WPI/G = 2.1 films as a function of pH. Each experimental point is represented by its c_g . Error bars show the $\pm 95\%$ confidence interval.

Table 3. OP of WPI/S = 1.2 Films at pH 7 and 9 at Different Concentrations

WPI/S = 1.2	pH 7 ^a	OP ^{b,c}	pH 9 ^a	OP ^{b,c}
low concentrated	7.7	3.0 ± 0.4	7.3	1.9 ± 0.4
c_g	11.7	2.4 ± 0.2	11.3	0.9 ± 0.1
high concentrated	15.7	2.2 ± 0.2	14.3	0.6 ± 0.1

^a % (w/w) WPI. ^b $\text{cm}^3 \mu\text{m}^2 \text{d kPa}$ ^c Mean values \pm standard deviation.

increased MC is an important parameter, due to the plasticizing effect of water on biopolymeric films (Slade et al., 1989; Arvanitoyannis and Biliaderis, 1998; Arvanitoyannis et al., 1994, 1996, 1997, 1998; Kalichevsky et al., 1992, 1993; Roos, 1995). Banerjee and Chen (1995) showed that WPI/G = 2 films at pH 7.3 had an MC of 25.1%. In the present study the MC was 20.5% for the WPI/G = 2.1 films formed at pH 7. The difference in the MC values was probably mostly due to the storage conditions of 55% RH that Banerjee and Chen (1995) used instead of the normal 50% RH applied in the present investigation. A higher RH imparts a higher MC in WPI films (Anker et al., 1999). Nevertheless, the results from Banerjee and Chen (1995) show that using G as a plasticizer in WPI films results in large MC values.

The increased WVP values for the WPI/G = 2.1 films are probably due to the increased MC for these films. This is in accordance with the work of Maté and Krochta (1996), who reported that WVP increases with increased MC for hydrophilic films, due to the plasticizing effect of the water. Several researchers have reported increased WVP values when G has been used as plasticizer instead of S. McHugh et al. (1994) showed that WPI/G = 1 films exhibited significantly higher WVP values than WPI/S = 1.7 films. Chick and Ustunol (1998) showed that casein-based films plasticized with G had higher WVP values than films plasticized with S when the same amounts of plasticizer were used.

Effect of the pH. When the pH increases, the OP decreases. This is shown in Table 3, where the OP values for the WPI/S = 1.2 films at pH 7 and 9 are presented. The oxygen molecules are believed to permeate through the protein phase and, since the protein matrix becomes denser at pH 9 (Figure 3c,f,i) compared with pH 7 (Figures 3a,d,g) the oxygen molecules find it more difficult to permeate at the higher pH value. Consequently, the OP decreases. This effect is similar to increasing the concentration, as previously discussed.

CONCLUSIONS

In this investigation valuable information regarding the relationship between the microstructure and the

mechanical and barrier properties of whey protein films is presented. The microstructure of the films was found to be dependent on three different parameters: the concentration, the plasticizers, and the pH. When the concentration increases, more aggregated structures are formed, with a denser protein matrix and larger pores. This affects the barrier properties, since the WVP increases and the OP decreases. When G is used as a plasticizer instead of S the overall microstructure is different, and the MC and WVP approximately doubled in value. When the pH increases from 7 to 9, a denser protein structure is formed, the strain at break increases, and the OP decreases. The transmission electron microscopy used in this study has proved to be an important tool for evaluating and interpreting the influence of the different parameters on the whey protein film microstructure.

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