

# Effects of pH and the Gel State on the Mechanical Properties, Moisture Contents, and Glass Transition Temperatures of Whey Protein Films

Martin Anker, Mats Stading,\* and Anne-Marie Hermansson

SIK—The Swedish Institute for Food and Biotechnology, P.O. Box 5401, SE-402 29 Göteborg, Sweden

The mechanical properties, moisture contents (MC), and glass transition temperature ( $T_g$ ) of whey protein isolate (WPI) films were studied at various pH values using sorbitol (S) as a plasticizer. The films were cast from heated aqueous solutions and dried in a climate chamber at 23 °C and 50% relative humidity (RH) for 16 h. The critical gel concentrations ( $c_g$ ) for the cooled aqueous solutions were found to be 11.7, 12.1, and 11.3% (w/w) WPI for pH 7, 8, and 9, respectively. The cooling rate influenced the  $c_g$ , in that a lower amount of WPI was needed for gelation when a slower cooling rate was applied. Both cooling rates used in this study showed a maximum in the  $c_g$  at pH 8. The influence of the polymer network on the film properties was elucidated by varying the concentration of WPI over and under the  $c_g$ . Strain at break ( $\epsilon_b$ ) showed a maximum at the  $c_g$  for all pH values, thus implying that the most favorable structure regarding the ability of the films to stretch is formed at this concentration. Young's modulus ( $E$ ) and stress at break ( $\sigma_b$ ) showed a maximum at  $c_g$  for pH 7 and 8. The MC and  $\epsilon_b$  increased when pH increased from 7 to 9, whereas  $T_g$  decreased. Hence,  $T_g$  values were  $-17$ ,  $-18$ , and  $-21$  °C for pH 7, 8, and 9, respectively.  $E$  and  $\sigma_b$  decreased and  $\epsilon_b$  and thickness increased when the surrounding RH increased. The thickness of the WPI films also increased with the concentration of WPI.

**Keywords:** *Whey protein; critical gel concentration; mechanical properties; moisture content; glass transition temperature; dynamic mechanical analysis*

## INTRODUCTION

Whey, the byproduct of cheese-making, is one of the biggest reservoirs of food protein remaining largely outside human consumption channels. The world whey output was  $\sim 120$  million tons in 1990, containing  $\sim 0.7$  million ton of relatively high value whey protein (Bylund, 1995), yet a considerable proportion of the total whey output is still wasted—the proportion of wastage was roughly 50% in 1989–1990. Hence, great efforts are being made to find new uses for whey protein, for example, as edible and biodegradable films. The potential applications of edible and biodegradable films are several: the films could be used together with paper, resulting in biodegradable packaging; the films could be used inside foods as a migration barrier between different layers, for example, in pies and confectionery; the films could function as controlled-release carriers for antioxidants and antimicrobial agents, as well as supplementing the nutritional value of foods. Consequently, the interest in the study of edible and biodegradable films has increased during the past decade, and current research on the properties of edible and biodegradable films has been outlined in recent reviews (Krochta et al., 1994; Anker, 1996; Callegarin et al., 1997; Gennadios et al., 1997; Guilbert et al., 1997; Krochta, 1997; Krochta and De Moulder-Johnston, 1997; Miller and Krochta, 1997; Shellhammer and Krochta, 1997; Cuq et al., 1998).

The present study on whey protein *films* was promoted by the work previously performed on  $\beta$ -lactoglobulin and whey protein *gels* in our laboratory. The findings from these studies have been used as a basis for producing films and for obtaining further data, which can be used in future food-packaging applications.  $\beta$ -Lactoglobulin is the dominating whey protein and tends to influence the behavior of whey protein *gels* (Hermansson, 1979, 1986; Stading and Hermansson, 1990, 1991; Langton and Hermansson, 1992; Stading et al., 1992, 1993). The microstructure and physical properties of whey protein gels are sensitive to thermal treatment, that is, heating and cooling rates, the amount of salt addition, pH, the concentration of whey protein, and the concentration of other additives such as plasticizers. The heating rate has therefore been held constant in this investigation, no salt has been added, and the ratio of whey protein concentration to sorbitol concentration has been held constant to eliminate the plasticizing effect of sorbitol. This allows a study of the other factors, the influence of which will be elucidated in this paper.

In a previous study the behavior of whey protein isolate (WPI) and  $\beta$ -lactoglobulin ( $\beta$ -Lg) films was studied using sorbitol (S) as a plasticizer (Anker et al., 1998). The pH and concentration of WPI,  $\beta$ -Lg, and S were varied, and the mechanical and barrier properties were elucidated. As for the whey protein *gels*, the dominating protein  $\beta$ -lactoglobulin influenced the behavior of whey protein *films*. It could also be seen that the WPI and  $\beta$ -Lg films showed different behavior at pH 7 and 8 compared to that at pH 9. Why the films behaved differently was not clear, but it could be due

\* Author to whom correspondence should be addressed [telephone +46 31 335 56 00; fax +46 31 83 37 82; e-mail mats.stading@sik.se].

to alkali-induced chemical changes in the polymer matrix at high pH values. Hence, the pH range between 7 and 9 was further studied in this paper.

Another interesting point that emerged from our previous study (Anker et al., 1998) was the importance of the critical gel concentration ( $c_g$ ). Some of the WPI and  $\beta$ -Lg films had concentrations that were below the  $c_g$ , according to Stading and Hermansson (1990), but still formed continuous films. This suggested that it was not necessary to reach  $c_g$  to obtain film with acceptable mechanical properties. Hence, the significance of  $c_g$  was investigated in this paper.

The  $c_g$  is the minimum concentration needed to form a gel under certain gelation process conditions. At the gel point, the protein network spans the whole system, and a gel is formed. Basically, the whey protein gelation includes two main reactions: heat-induced denaturation and aggregation (Foegeding, 1997; Verheul et al., 1998). The denaturation temperatures of  $\beta$ -lactoglobulin have been 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). The  $\beta$ -lactoglobulin denaturation causes partial protein unfolding and changes in the molecular structures. This leads to exposure of amino acids or peptide segments, which form intermolecular complexes by covalent (disulfide) or noncovalent (hydrogen bonds, hydrophobic interactions, or electrostatic bonds) interactions. The relative significance of the disulfide bonds has been widely discussed in the past, because they are believed to play a role in the heat-induced aggregation and gelation of  $\beta$ -lactoglobulin (Britten, 1997; Hoffman, 1997). Still, the extent of the disulfide bonds' relative contribution of the overall aggregation and gelation process is unclear (McSwiney et al., 1994). The second reaction in the whey protein gelation is further aggregation of the gel matrix, which in this study occurs during the cooling and simultaneous drying of the cast film-forming solution.

The glass transition temperature ( $T_g$ ) has been an important parameter in the study of synthetic polymers.  $T_g$  is also proving to be of interest for edible and biodegradable polymers, because it influences the use of these materials. For example, it is expected that the permeation of gas and vapor molecules through a film will be higher above  $T_g$ , where polymer chains are more mobile (McHugh and Krochta, 1994a). Above  $T_g$ , polymeric materials exist in a soft, rubbery state, which impairs barrier properties, whereas below  $T_g$ , polymers assume a glassy, low-permeable state (Cherian et al., 1995). Generally,  $T_g$  is increased by increasing the amount of stiff chains and bonds, bulky side groups, cross-linking between chains, and the degree of crystallinity, whereas  $T_g$  is decreased when the amount of low molecular plasticizers is increased (Levine, 1983; Rogers, 1985). In the case of edible and biodegradable films, water acts as a plasticizer depressing  $T_g$  (Slade et al., 1989). Addition of small quantities of water to these materials leads to segmental mobility of chains, which in turn produces structural relaxation at a decreased temperature. Water is further considered the most important plasticizer, because it is the most ubiquitous material and governs the workability and behavior of food materials (Roos, 1995). Water is also one of the plasticizers with a very low molecular weight and an extremely low  $T_g$  (Roos, 1995). Lillie and Gosline (1993) pointed out that the  $T_g$  of proteins, especially at a low

**Table 1. Concentrations and Properties of Proteins in the WPI Powder**

| protein                | WPI <sup>a</sup> (%) | molecular mass <sup>b</sup> (Da) | cysteine groups <sup>b</sup> |
|------------------------|----------------------|----------------------------------|------------------------------|
| $\beta$ -lactoglobulin | 74                   | 18600                            | 2 (1-SH)                     |
| $\alpha$ -lactalbumin  | 18                   | 14200                            | 4                            |
| bovine serum albumin   | 6                    | 66000                            | 17 (1-SH)                    |
| immunoglobulins        | 2                    | 150000–1000000                   | 32                           |

<sup>a</sup> From MD Foods Ingredients (1997). <sup>b</sup> From Kinsella and Whitehead (1989).

water content, may occur over a wide temperature range. However, few  $T_g$  data are available for proteins (Roos, 1995). Fasold et al. (1995) showed that the permeation of oxygen was lower below the  $T_g$  in whey protein film and that the  $T_g$  decreased with increasing moisture content. The authors also showed that the  $T_g$  decreased with decreasing molecular size of the plasticizer when sorbitol, glycerol, and propylene glycol were used.

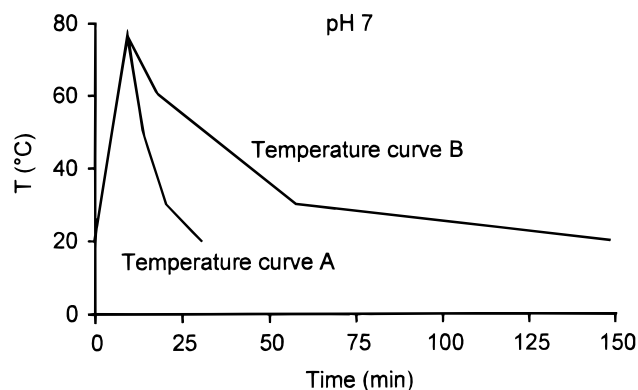
The aim of this paper was to study the mechanical properties, moisture content (MC), and  $T_g$  of films made of WPI at pH 7, 8, and 9. The  $c_g$  was investigated for the WPI film-forming solutions at the different pH values. The effect of varying the concentration of WPI over and under the  $c_g$  was studied to elucidate the importance of the gel network. Both large and small deformation measurements were used to evaluate and interpret the results.

## 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). Sorbitol (S) (>98% purity) was used as a plasticizer and was obtained from Sigma Chemical Co. (St. Louis, MO). The concentration and properties of proteins in the WPI powder are listed in Table 1.

**Dynamic Oscillatory Measurements.** Dynamic oscillatory measurements were performed in a Bohlin VOR Rheometer (Bohlin Rheology, Chichester, U.K.) to find the  $c_g$  at pH 7, 8, and 9. Ten milliliters of solution was poured in a double-gap (DG) measuring system. The DG system was used to obtain the highest possible sensitivity. 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  $1.6 \times 10^{-3}$ , 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. Because the kinetics, that is, the heating and cooling rate, influence the microstructure and physical properties of whey protein gels, the heating rate was held constant and two different cooling rates were applied. In Figure 1, the temperature curves show the two cooling rates applied at pH 7. The corresponding temperature curves for pH 8 and 9 were similar. Temperature curve A, with the film-formation cooling rate, is a simulation of the temperature increase and decrease of the film-formation process. Temperature curve B, with the slower cooling rate, was applied to elucidate how the  $c_g$  was affected. 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  $\beta$ -lactoglobulin. Different maximum temperatures for each pH were used, because the denaturation temperature, and thus the gelling, varies with pH (Hegg, 1980). Thereafter the solutions were allowed to cool



**Figure 1.** Temperature curves A and B as a function of time, represented by pH 7. Temperature curve A, with the film-formation cooling rate, is a simulation of the temperature increase and decrease during the film-formation process. Temperature curve B, with the slower cooling rate, was applied to elucidate how the  $c_g$  was affected.

**Table 2. Experimental Design**

| pH 7,<br>% (w/w) WPI    | pH 8,<br>% (w/w) WPI    | pH 9,<br>% (w/w) WPI    |
|-------------------------|-------------------------|-------------------------|
| 7.7                     | 8.1                     | 7.3                     |
| 9.7                     | 10.1                    | 9.3                     |
| <b>11.7<sup>a</sup></b> | <b>12.1<sup>a</sup></b> | <b>11.3<sup>a</sup></b> |
| 13.7                    | 14.1                    | 13.3                    |
| 15.7                    | 16.1                    | 14.3                    |

<sup>a</sup> Critical gel concentration.

to 20 °C, with the steepest cooling rate in the beginning. Hence, the cooling rates at pH 7 were subsequently  $-6$ ,  $-3$ , and  $-1$  °C/min for temperature curve A and  $-1.9$ ,  $-0.8$ ,  $-0.1$  °C/min for temperature curve B. This resulted in cooling times for temperature curves A and B of  $\approx 20$  and 140 min, respectively.

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  $j = -1$ . The phase angle ( $\delta$ ) 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 temperature curves A and B. The  $\delta$  at 1 Hz was recorded for each concentration tested. Linear regression was used to estimate the critical concentration as  $c_g$  ( $\delta = 45^\circ$ ) with a 95% confidence level.

**Film Formation.** The concentration of WPI was varied over and under the  $c_g$  according to the experimental design displayed in Table 2. The ratio of WPI concentration to S concentration was held constant (WPI/S = 1.2). 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  $\approx 6$  °C/min. A total of 15.4 g of solution was cast onto each polystyrene Petri dish, with an internal diameter of 14 cm, to be able to evaluate if the variation in the WPI concentration influenced the thickness of the films. The cast solutions were allowed to cool and dry at room temperature for  $\approx 4$  h and were then dried in a climate-controlled room at 23 °C and 50% relative humidity (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). Films were preconditioned in a climate chamber at 23 °C and 50, 63, and 82% RH, respectively, for at least 48 h prior to all testing. For further details regarding the film formation, see Anker et al. (1998).

**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. At least eight replicates of each experimental point were evaluated. MC was calculated as the percentage of weight loss based on the original weight, in accordance with ASTM D664-94.

**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. The mechanical properties at the large and small deformation analyses were calculated using the average thickness for each film replicate.

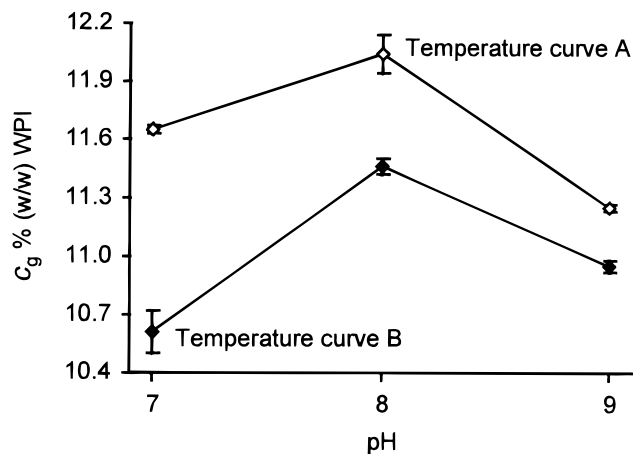
**Mechanical Properties.** A texture analyzer (model TA-XT2, Stable Micro Systems, Godalming, England) was used to determine mechanical properties at large deformations in accordance with ASTM D882-91. The films were tested in a climate-controlled room at 23 °C and 50% RH and in a climate chamber at 23 °C and 63 and 82% 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 ( $\sigma_y$ ), stress at break ( $\sigma_b$ ), and strain at break ( $\epsilon_b$ ) 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.

**Dynamic Mechanical Analysis.** The small deformation analysis of the films was performed in tension in a Rheometrics solids analyzer, RSA-II (Rheometrics Scientific, Piscataway, NJ). The tested filmstrips were 35 mm long and 4 mm wide and clamped in the instrument with the initial grip separation 23.4 mm. The films were subjected to a sinusoidal strain on top of a static deformation, the sum of these being nondestructive. The magnitude and phase of the resulting sinusoidal stress were measured, together with the total elongation ( $\Delta L$ ) of the filmstrip during the measurements. The storage modulus ( $E'$ ) and the loss modulus ( $E''$ ) were calculated together with the phase angle ( $\delta$ ):  $\tan \delta = E''/E'$ .  $T_g$  was measured as the temperature of the  $\tan \delta$  peak. The testing was conducted at a constant frequency of 1 Hz and a strain of 0.01% and over a temperature range of  $-60$  to 100 °C, at a heating rate of 5 °C/min. The measurements of each experimental point were done at least in triplicates.

The RSA-II instrument was equipped with a humidity control system designed at SIK (Stading, 1998). The films were conditioned at 20 °C and 50% RH for 20 min, which ensured accurate measurements, because the transfer from the climate chamber to the RSA-II instrument exposed the films to ambient RH. The conditioning for 20 min set the films in equilibrium with their surroundings, which could be seen by constant  $\Delta L$  and  $E'$  in the RH sweeps performed. The temperature sweeps were then performed, after the films had been covered with hydrophobic grease. The method of covering the films with grease prevents moisture loss during temperature sweeps, which ensures correct measurements of the material properties (Stading, 1998). Increased  $\Delta L$  values with increasing temperature were taken as the indicator of sufficient moisture retention.

## RESULTS AND DISCUSSION

The results from the different pH values are presented side by side in this study. First, the quantitative analysis of the results of the dynamic oscillatory measurements, and consequently the  $c_g$ , is presented. Second, the quantitative analysis of the results of the MC and thickness of the films are shown. The MC of the films is of vital importance, because water acts as a plasticizer for biopolymeric films and influences other properties, such as  $\epsilon_b$  and  $T_g$ , and is therefore presented at an early stage. Third, the quantitative analysis of the results of the mechanical properties at large deformation is presented. The influence of increased RH, and



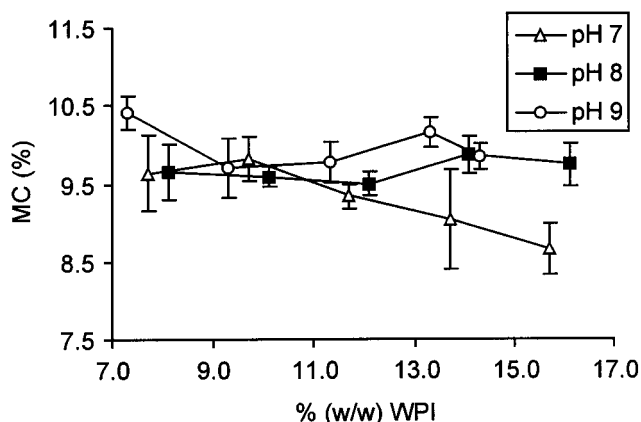
**Figure 2.**  $c_g$  as a function of pH. Error bars show  $\pm 95\%$  confidence interval.

consequently of increased MC, is also elucidated. Finally, the quantitative analysis of the results of the dynamic mechanical analysis, and thereby the  $T_g$ , at small deformation is shown.

**Critical Gel Concentration ( $c_g$ ).** Figure 2 shows the  $c_g$  for temperature curves A and B and, consequently, the two different cooling rates applied. The  $c_g$  values for temperature curve A, with the simulated film-formation cooling rate, are 11.7, 12.1, and 11.3% (w/w) WPI for pH 7, 8, and 9, respectively. When temperature curve B and the slower cooling rate were applied, the same maximum at pH 8 was shown, but a lower amount of WPI was needed to form a gel. Because gelation requires a critical minimum time for the reaction to occur, the slower cooling rate exposes the molecules to the higher temperatures for a longer time, which enhances the gelation and lowers the required amount of WPI. The maximum  $c_g$  at pH 8 indicates structural changes occurring in the gel network above pH 8. Similar observations with regard to pH were made in a previous study (Anker et al., 1998). The different  $c_g$  values in the present investigation were determined by varying the concentration within a broad range to localize the area of interest and, thereafter, by extensive measurements varying the concentration of WPI in steps of 0.1% (w/w) around the final  $c_g$ .

Few very accurate  $c_g$  data are available for whey proteins. Mulvihill et al. (1990) showed that no self-supporting gel could be formed at pH 8 with a concentration of 10% (w/v)  $\beta$ -lactoglobulin. Renard and Lefebvre (1992) showed that the  $c_g$  of  $\beta$ -lactoglobulin increased when pH was increased from the isoelectric point, that is, around pH 5.2, to 9. However, one should bear in mind the different techniques used in this latter investigation and the present study. Renard and Lefebvre (1992) heated the different concentrations for 1 h at a temperature as high as 100 °C and thereafter tilted the test tubes after 24 h. When the meniscus did not deform upon tilting, the system was considered to have gelled.

Another characteristic seen in Figure 2 is the difference between the  $c_g$  for each pH value, which decreases with increased pH values. The differences are 1, 0.6, and 0.3% (w/w) WPI at pH 7, 8, and 9, respectively. Stading and Hermansson (1990) pointed out that an alkali denaturation may disturb the fine-stranded network formation at pH 9. Britten (1997) also stated that moving the pH far from the isoelectric point increases charge repulsion, which in turn may disturb the ag-



**Figure 3.** MC of WPI films as a function of WPI concentration. Error bars show  $\pm 95\%$  confidence interval.

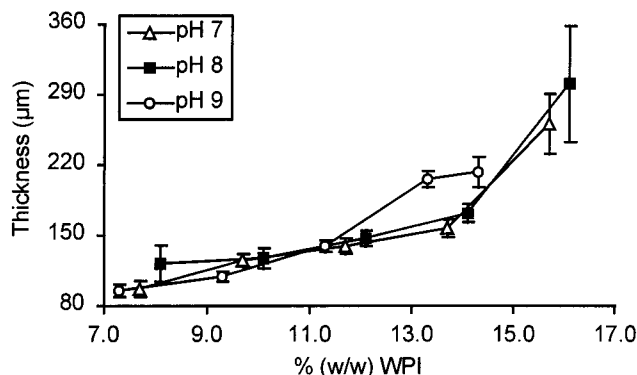
gregation process involved in gelation. These aspects may explain why the difference between the two cooling rates decreases with increasing pH.

Because one aim of this paper was to study the influence of varying the concentration of WPI over and under the  $c_g$ , the experimental design in Table 2 was chosen. The concentration of WPI was varied 2 and 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 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 over the  $c_g$ .

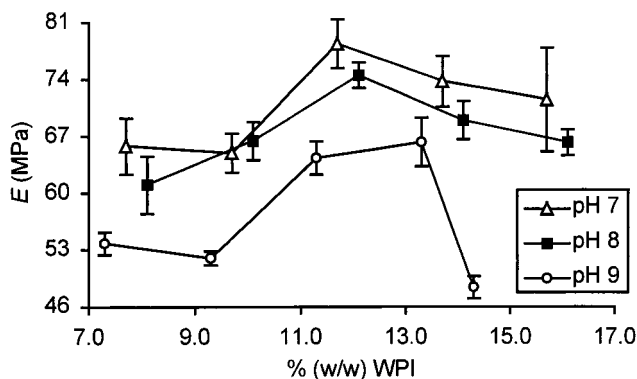
**Moisture Content and Film Thickness.** The MC in the WPI films was evaluated, and the quantitative result is presented in Figure 3. It can be seen that the MC increases slightly when the pH increases. However, it is not clear why the MC is higher at pH 9 than at pH 7. One explanation could be that, at a higher pH value, more charged molecules are present, which have a higher ability to adsorb water and thus increase the MC in the films. This result is in agreement with previous findings of casein films, in which the water content increased when pH increased from 7 to 9 (Frinault et al., 1997). It can also be seen that the MC is relatively constant at pH 8 and 9, whereas at pH 7 the MC slightly decreases when the concentration of WPI increases. Still, the difference in MC is rather small between the different pH values. For example, at experimental point  $c_g$  the MC values are 9.3 and 9.8% for pH 7 and 9, respectively.

In Figure 4, the plot shows how the thickness increases when the concentration of WPI increases. At high concentrations of WPI, less water is present, and during the drying process of the films, water evaporates and thicker films are produced. Another interesting distinction is that at high concentrations, >15% (w/w) WPI, a large increase in the thickness is observed. This is due to the enhanced and fast gelling that occurs at high concentrations of WPI. The influence of the high concentrations is being further investigated in a separate study.

**Mechanical Properties at Large Deformations.** Four mechanical properties were derived from the large deformation tensile test: Young's modulus ( $E$ ), stress at maximum force ( $\sigma_y$ ), stress at break ( $\sigma_b$ ), and strain at break ( $\epsilon_b$ ). The result from  $\sigma_y$  is almost identical with  $\sigma_b$ ; thus, only the latter is presented in this paper. In



**Figure 4.** Thickness of WPI films as a function of WPI concentration. Error bars show  $\pm 95\%$  confidence interval.



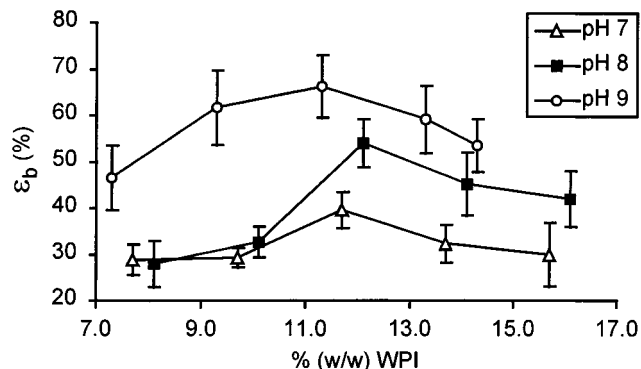
**Figure 5.** Young's modulus ( $E$ ) of WPI films as a function of WPI concentration. Error bars show  $\pm 95\%$  confidence interval.



**Figure 6.** Stress at break ( $\sigma_b$ ) of WPI films as a function of WPI concentration. Error bars show  $\pm 95\%$  confidence interval.

Figures 5 and 6, the changes in  $E$  and  $\sigma_b$  are presented together with the variations in WPI concentrations over and under the  $c_g$ . The plots show a maximum at the  $c_g$  for pH 7 and 8. This implies that the most favorable structures are formed at 11.7 and 12.1% (w/w) WPI at pH 7 and 8, respectively. However, at pH 9 a maximum is still shown, but at a higher concentration than  $c_g$ , that is, at 13.3% (w/w) WPI. Why the films show this different behavior at different pH values is not clear, but it may be due to structural changes in the polymer matrix at alkaline pH. At high pH values, the proteins become more negatively charged. This results in greater electrostatic repulsion, which disturbs protein-protein interactions (Zirbel and Kinsella, 1988).

The decrease in  $E$  and  $\sigma_b$  above the  $c_g$  at pH 7 and 8, and above 13.3% WPI at pH 9, indicates that, when the concentration of WPI increases, different network structures are formed. This effect is more pronounced at pH 9, where the  $E$  and  $\sigma_b$  at 14.3% (w/w) WPI are markedly



**Figure 7.** Strain at break ( $\epsilon_b$ ) of WPI films as a function of WPI concentration. Error bars show  $\pm 95\%$  confidence interval.

decreased. Moreover, it was very difficult to cast films at concentrations higher than 15.7, 16.1, and 14.3% (w/w) WPI at pH 7, 8, and 9, respectively. A final distinction is that below the  $c_g$ , both  $E$  and  $\sigma_b$  are decreased. These results show that films are formed below  $c_g$ . The films are weaker but still show acceptable mechanical properties. However, the best mechanical properties seem to be at, or above, the  $c_g$ .

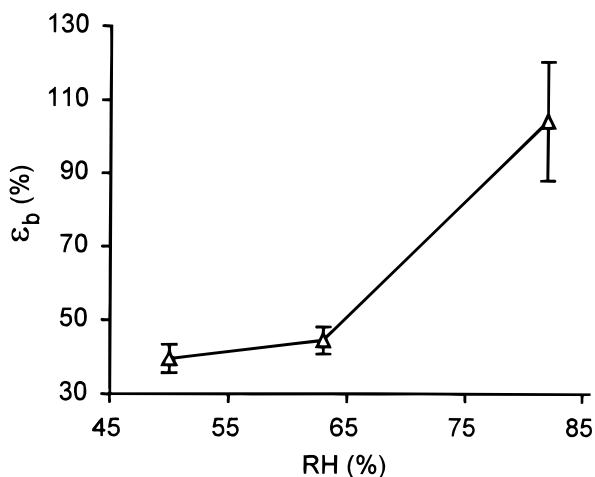
Figure 7 shows a maximum for  $\epsilon_b$  at the  $c_g$  for all pH values. This implies that the most favorable structure regarding the ability of the films to stretch is formed at this concentration. Above the  $c_g$ , the protein network structure is probably less ordered, due to the enhanced and fast gelling that occurs at high concentrations. There is insufficient time for the unfolded protein molecules to rearrange in the most favored structure prior to aggregation. Hermansson (1978) stated that, when denaturation and random aggregation occur simultaneously, protein gels with poor elasticity are formed. It can also be seen that the curves at pH 7 and 8, compared to pH 9, show a slightly different behavior. This also indicates a change in the polymer matrix above pH 8, as previously discussed.

Another clear distinction from Figure 7 is that  $\epsilon_b$  increases as the pH increases. This is partly explained by the plasticizing effect of water and partly explained by the occurrence of structural changes. One should bear in mind that the ratio of WPI to S is constant in the present investigation and, consequently, that the concentration of S does not affect the  $\epsilon_b$ . However, it is known that water acts as a plasticizer for biopolymeric films (Slade et al., 1989; Arvanitoyannis and Biliaderis, 1998; Arvanitoyannis et al., 1994, 1996, 1997, 1998; Kalichevsky et al., 1992, 1993; Roos, 1995). Increasing the amount of plasticizer weakens the intermolecular forces between adjacent polymer chains. This results in films with lower tensile strength and greater elongation (Gennadios et al., 1994). The influence of the plasticizing effect of water and the occurrence of structural changes are elucidated in Table 3. The upper part of Table 3 shows how the MC and  $\epsilon_b$  increase with pH at each  $c_g$ . The lower part of Table 3, at constant pH 7 and 11.7% (w/w) WPI, presents how an increased surrounding RH affects the properties. A RH of 63% results in films with a MC of 10.9% and a  $\epsilon_b$  of 44%. This should be compared with the MC and  $\epsilon_b$  values in the upper part of Table 3. For example, at pH 9, the MC and  $\epsilon_b$  are 9.8 and 66%, respectively. The latter result shows that even when the MC is lower, the  $\epsilon_b$  is higher. If the  $\epsilon_b$  of the films had been the results of only the plasticizing effect of the MC, they would have had a lower  $\epsilon_b$ . However, this is not

**Table 3. MC, Thickness, and Mechanical Properties of WPI Films Preconditioned at Different RH Values**

|                              | pH | concn of WPI % (w/w) | RH <sup>a</sup> (%) | MC <sup>b</sup> (%) | thickness <sup>b</sup> ( $\mu$ m) | $\epsilon_b^{b,c}$ (%) | $E^{b,c}$ (MPa) | $\sigma_b^{b,c}$ (MPa) |
|------------------------------|----|----------------------|---------------------|---------------------|-----------------------------------|------------------------|-----------------|------------------------|
| constant RH                  | 7  | 11.7                 | 50                  | 9.3 $\pm$ 0.2       | 139 $\pm$ 8                       | 40 $\pm$ 4             | 78 $\pm$ 3      | 2.9 $\pm$ 0.1          |
|                              | 8  | 12.1                 | 50                  | 9.5 $\pm$ 0.2       | 147 $\pm$ 8                       | 54 $\pm$ 5             | 74 $\pm$ 2      | 2.9 $\pm$ 0.2          |
|                              | 9  | 11.3                 | 50                  | 9.8 $\pm$ 0.2       | 139 $\pm$ 6                       | 66 $\pm$ 7             | 64 $\pm$ 2      | 2.9 $\pm$ 0.1          |
| constant pH and concn of WPI | 7  | 11.7                 | 50                  | 9.3 $\pm$ 0.2       | 139 $\pm$ 8                       | 40 $\pm$ 4             | 78 $\pm$ 3      | 2.9 $\pm$ 0.1          |
|                              | 7  | 11.7                 | 63                  | 10.9 $\pm$ 2.5      | 144 $\pm$ 5                       | 44 $\pm$ 4             | 37 $\pm$ 1      | 1.3 $\pm$ 0.1          |
|                              | 7  | 11.7                 | 82                  | 13.2 $\pm$ 2.7      | 180 $\pm$ 9                       | 104 $\pm$ 16           | 3 $\pm$ 1       | 0.2 $\pm$ 0.0          |

<sup>a</sup> Films were preconditioned at 23 °C and 50, 63, and 82% RH for at least 48 h prior to all testing. <sup>b</sup> Mean values  $\pm$  95% confidence interval. <sup>c</sup> The tensile test was conducted at 23 °C at each RH.



**Figure 8.** Strain at break ( $\epsilon_b$ ) for 11.7% (w/w) WPI films at pH 7 as a function of RH. Films were preconditioned at 23 °C and 50, 63, and 82% RH for at least 48 h prior to testing. The tensile test was conducted at 23 °C at each RH. Error bars show  $\pm$ 95% confidence interval.

the case, and some kind of structural change is likely to occur in the polymer matrix as the pH increases, which makes the fine-stranded network more flexible at higher pH values. This result is in agreement with previous findings of casein films, in which the elongation increased when pH increased from 7 to 9 (Frinault et al., 1997).

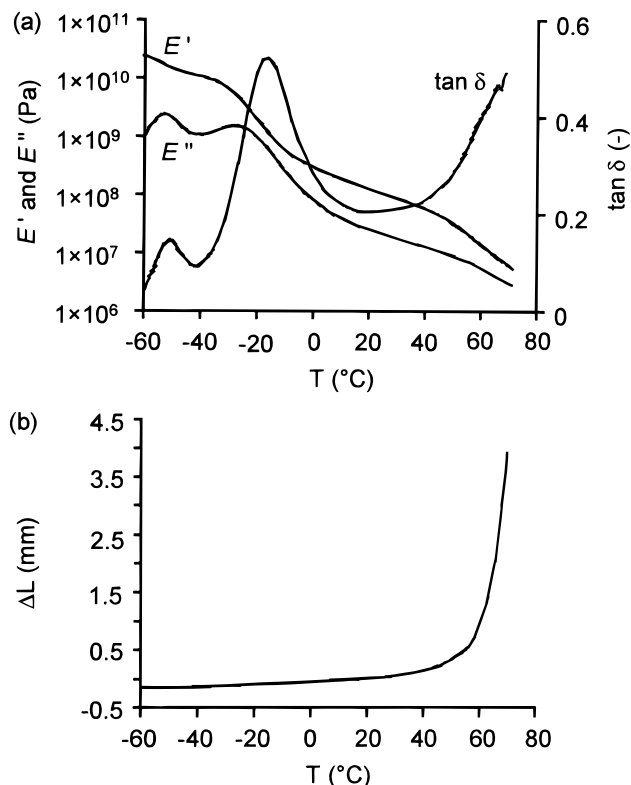
The properties of the WPI films are highly affected when the surrounding RH increases, as presented in Table 3. The effect of the increased RH is exemplified in Figure 8, where the  $\epsilon_b$  is plotted as a function of RH. Miller et al. (1997) presented similar results for WPI films, showing an increased  $\epsilon_b$  as RH increased. Several other studies have shown that both RH and temperature exhibit exponential effects on the permeability of biopolymeric films (Arvanitoyannis et al., 1997; McHugh and Krochta, 1994b; McHugh et al., 1993, 1994). McHugh and Krochta (1994b) showed an exponential effect on the water vapor permeability of whey protein films as RH increased. Similar results are presented in Table 3 and Figure 8 for the mechanical properties.  $E$  and  $\sigma_b$  decrease and  $\epsilon_b$  increases when the RH increases. For example,  $E$  decreases from 78 MPa at 50% RH, to 37 MPa at 63% RH, and finally to 3 MPa at 82% RH. Another distinction is that the thickness of the films increased when the RH increased. This is, of course, due to the increased MC in the films. This also implies that if the barrier properties, for example, the water vapor permeability, had been tested in this paper, exponential behavior similar to that presented by McHugh and Krochta (1994b) would have been expected.

The behavior of whey protein gels at high pH values has been studied in several investigations. Shimada and

Cheftel (1988) showed that the firmness, defined as stress at 40% compression, of whey protein gels decreased when pH increased from 6.5 to 9.5. This was explained by more negatively charged proteins as pH increased. It resulted in greater electrostatic repulsion and less protein–protein interaction, and consequently, weaker gels formed (Shimada and Cheftel, 1988; Zirbel and Kinsella, 1988; Xiong and Kinsella, 1990). This effect may also explain the decreased  $E$  of the WPI films when pH increased from 7 to 9, because the films in this study were formed from gels (upper part of Table 3). The pH has also been shown to influence the elasticity of whey protein gels. An increase in elasticity of heat-induced whey protein gels at increased pH values was suggested to be due to an increased number of intermolecular disulfide (S–S) bonds (Shimada and Cheftel, 1988). As pH increases to ~8, thiol/disulfide (SH/S–S) interchange or thiol/thiol (SH/SH) oxidation reactions may occur upon heating, and intermolecular S–S bonds can be formed (DeWit and Klarenbeek, 1983; Li-Chan, 1983; Mangino et al., 1987; Kinsella and Whitehead, 1989). Monahan et al. (1995) showed a lower SH content of whey protein solutions at pH 9 and 11, relative to the solutions at lower pH values. This suggested that SH–SH oxidation to S–S occurred in addition to SH/S–S interchange reactions. The decrease in SH content at alkaline pH values may also be due to degradation reactions. In alkaline media, cystine and cysteine residues may be converted to degradation products such as H<sub>2</sub>S and dehydroalanine (Watanabe and Klostermeyer, 1976).

**Glass Transition Temperature ( $T_g$ ).** The thermal and rheological behavior of the small deformation test is shown in Figure 9, represented by a 12.1% (w/w) WPI film at pH 8. Figure 9a shows a sharp peak in the  $\tan \delta$  around  $-18$  °C and a drop in  $E'$ , indicating the glass transition temperature ( $T_g$ ). The  $T_g$  decreases as the MC increases from pH 7 to 9, as presented in Table 4. Several researchers have shown the plasticizing effect of water depressing  $T_g$  (Slade et al., 1989; Kalichevsky et al., 1992, 1993; Arvanitoyannis and Biliaderis, 1998; Arvanitoyannis et al., 1994, 1996, 1997, 1998; Gontard and Ring, 1996). Arvanitoyannis et al. (1997) observed decreased  $T_g$  values as the MC increased for gelatin/soluble starch/water blends, which was explained by increased hydrogen bonding between the starch–water chains and decreased intra- and intermolecular hydrogen bonding between the starch chains. This leads to lower interaction energy between the biopolymer chains and increased segmental mobility, which leads to structural relaxation at decreased temperatures.

It has also been shown that addition of low molecular plasticizers, such as sorbitol, decrease  $T_g$  in blends with corn starch/sorbitol/water (Arvanitoyannis et al., 1996). The authors implied that the increase in sorbitol



**Figure 9.** Representative DMA plot of a 12.1% (w/w) WPI film at pH 8. Storage modulus ( $E'$ ), loss modulus ( $E''$ ), phase angle ( $\tan \delta$ ) (a), and elongation ( $\Delta L$ ) (b) as a function of temperature.

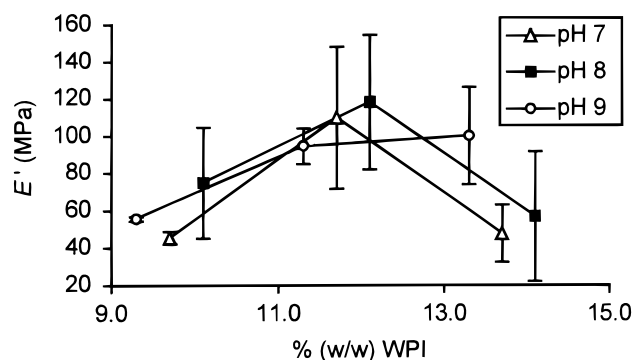
**Table 4.**  $T_g$  and MC of WPI Films at pH 7, 8, and 9

| pH | MC <sup>a</sup> (%)    | $T_g^{b,f}$ (°C)     |
|----|------------------------|----------------------|
| 7  | 9.4 ± 0.1 <sup>c</sup> | -17 ± 2 <sup>c</sup> |
| 8  | 9.6 ± 0.1 <sup>d</sup> | -18 ± 3 <sup>d</sup> |
| 9  | 9.8 ± 0.1 <sup>e</sup> | -21 ± 2 <sup>e</sup> |

<sup>a</sup> Mean values ± 90% confidence interval. <sup>b</sup> Mean values ± 95% confidence interval. <sup>c</sup> Mean values from 9.7, 11.7, and 13.7% (w/w) WPI films. <sup>d</sup> Mean values from 10.1, 12.1, and 14.1% (w/w) WPI films. <sup>e</sup> Mean values from 9.3, 11.3, and 13.3% (w/w) WPI films. <sup>f</sup> Independently of applied grease during DMA measurements.

concentration would allow more moisture uptake. This was shown by Anker et al. (1998), in that increasing the concentration of sorbitol indeed increased the MC. The authors implied that this was due to the six hydroxyl groups that sorbitol contains, which can readily bond with water molecules and increase the water-holding capacity of WPI films. Arvanitoyannis et al. (1996) also showed a large impact on the  $T_g$  when the concentration of sorbitol, and thus the MC, varied.  $T_g$  increased from -18.9 to -4.4 °C when the concentration of sorbitol decreased from 28 to 24% (w/w). In this study,  $T_g$  increased from -21 to -17 °C when the MC decreased from 9.8 to 9.4%. This variation in  $T_g$  was probably due to the small variation in MC. This is in agreement with Roos (1995), who stated that even a very small increase in the water content leads to significant depression of the  $T_g$ .

Another interesting point is the  $\Delta L$  of the WPI films when the temperature is raised (Figure 9b). From -60 to 20 °C,  $\Delta L$  increases ~0.1–0.15 mm. This is due to normal heat expansion of the WPI films. Subsequently, when the temperature is raised from 20 to 60 °C,  $\Delta L$  increases exponentially, and around 65 °C the dynamic mechanical analysis (DMA) instrument maximum 4 mm



**Figure 10.** Storage modulus ( $E'$ ) as a function of WPI concentration. Each  $E'$  value is a mean of at least three replicates. Error bars show ± standard deviation.

elongation limit is reached. The effect of increasing the temperature at a certain MC is thus equivalent to increasing the surrounding RH at a constant temperature (Biliaderis, 1990).

The peak in  $\tan \delta$  around -50 °C is caused by the hydrophobic grease used to prevent moisture loss in the DMA measurements (Stading, 1998). Consequently, the small increase in  $E'$  below -50 °C and the peak in  $E''$  at this temperature interval may be derived from this grease. Nevertheless, the grease has proved to work well at the temperature interval of -40 to 100 °C used in this study.

In Figure 10, the changes in  $E'$  at 23 °C are presented together with the variations in WPI concentration over and under the  $c_g$ . The plot verifies the behavior at the different pH values seen at the large deformation measurements in Figures 5 and 6. The  $E'$  shows similar behavior at pH 7 and 8, but dissimilar at pH 9, as the concentration of WPI varies over and under  $c_g$ .

**Conclusions.** The mechanical properties of the WPI films are greatly influenced by the concentration of WPI over and under the  $c_g$ . Strain at break ( $\epsilon_b$ ) shows a maximum at the  $c_g$  for all pH values, thus implying that the most favorable network structure regarding the ability of the films to stretch is formed at  $c_g$ . It can also be seen that the films' other mechanical properties show a different behavior at pH 7 and 8, compared to pH 9, as the concentration of WPI varies over and under the  $c_g$ . Why the films show this different behavior at different pH values is not clear, but it may be due to alkali-induced chemical changes in the polymer matrix at high pH values. The cooling rate influences the  $c_g$ , in that a lower amount of WPI is needed for gelation when a slower cooling rate is applied. Both cooling rates used in this study showed a maximum in the  $c_g$  at pH 8. The MC and  $\epsilon_b$  increased when pH increased from 7 to 9, whereas the  $T_g$  decreased. Hence, it is possible to tailor-make specific properties of the WPI films by varying the pH and MC. The MC is controlled by varying the concentration of plasticizer and the surrounding RH and temperature during drying. The results regarding the mechanical properties can all be related to the polymer network structures formed under different conditions. Therefore, further research is needed to fully understand the changes in the structural behavior that occur at different pH values and over and under the  $c_g$ . Microscopic techniques could be used to further study the network structural behavior presented in this work.

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