# Heat-Induced Conformational Changes in Whey Protein Isolate and Its Relation to Foaming Properties

Haiming Zhu and Srinivasan Damodaran*

Department of Food Science, University of Wisconsin—Madison, 1605 Linden Drive, Madison, Wisconsin 53706

Heat-induced changes in the physicochemical properties of whey protein isolate (WPI) have been studied. WPI (5%) heated at 70 °C underwent rapid conformational changes within 1 min. The aperiodic structure content increased primarily at the cost of β-sheet structure. The hydrophobic character, as measured by changes in the pH–solubility profile and the solubility profile at pH 4.6 in NaCl solutions, of the protein surface increased. However, the surface hydrophobicity, as measured by the *cis*-parinaric acid binding method, decreased. In contrast, WPI (9%) heated at 90 °C did not exhibit significant changes in the secondary structure content. The surface hydrophobicity decreased, and only minimal changes in the hydrophobic character of the protein surface occurred. The specific viscosity and gel electrophoretic data indicated that the majority of proteins in WPI heated at 90 °C were polymerized via sulfhydryl–disulfide interchange reactions, whereas such polymerization was minimal in the case of WPI heated at 70 °C. Studies on the foaming properties showed that WPI heated for 1 min at 70 °C possessed better foamability and foam stability than the other heat-treated samples. The improvement in foaming properties was not only affected by conformational changes but, more importantly, by the ratio of monomer to polymeric protein species present in WPI. Maximum foam stability was observed when the ratio of monomer to polymer was 40:60, whereas maximum foamability occurred at a 60:40 ratio. The results suggested that while monomeric proteins contributed to foamability, the polymeric species contributed to foam stability.

## INTRODUCTION

The growing demand in the food industry for functionally superior and nutritionally excellent novel proteins provides an opportunity for increasing the utilization of whey proteins in formulated food products. Although whey proteins are nutritionally excellent, their functional behavior in food systems, especially in foam and emulsion-type food products, does not meet the expectations. In general, several factors affect the functional properties of food proteins. These include intrinsic properties such as amino acid sequence and composition, secondary and tertiary structures, hydrophilic/hydrophobic character of the protein surface, net charge and charge distribution, and molecular rigidity/flexibility of the protein and extrinsic factors such as pH, ionic strength, temperature, and interactions with other food components.

Several studies have shown that the functional properties of whey protein concentrates (WPC) are affected by the method of isolation (de Wit et al., 1986; Richert et al., 1974; Richert, 1979; de Vilbiss et al., 1974). Morr (1985) reported that the foaming properties of four reference WPCs prepared from pasteurized and nonpasteurized acid casein whey and Cheddar cheese whey using ultrafiltration/diafiltration and spray-drying processes were very different. These differences were attributed to differences in protein and nonprotein composition as well as to differences in structural states of proteins.

Several studies also have shown that heating of WPC at moderate temperatures improved its foaming properties, whereas severe heat treatment resulted in impairment of foaming properties (Richert et al., 1974; Haggett, 1976; de Vilbiss et al., 1974; Cooney, 1974). Although it was intuitively understood that the extent of changes in the conformation and other intrinsic properties of proteins was responsible for the observed differences in the foaming properties of moderately and severely heat-treated WPC, the precise relationships between changes in physicochemical properties and foaming properties were not elucidated. In the present study, whey protein isolate was heat denatured at moderate and high temperatures; the changes in physicochemical properties, such as surface hydrophobicity, pH–solubility profile, solubility profile in salt solutions, secondary structure content, and hydrodynamic properties, were studied and the influence of such changes on foaming properties of whey protein isolate was elucidated.

## MATERIALS AND METHODS

Commercial whey protein isolate (BiPRO) prepared by an ion-exchange process was obtained from Le Sueur Isolates Co., Le Sueur, MN. According to the manufacturer, the typical composition of this WPI on dry basis was 95% protein, <1% ash, <1% fat, <1% lactose, and <5% moisture.

WPI solutions were prepared fresh with ultrapure water obtained from a Milli-Q Plus water purification system with a resistivity of 18.2 mΩ·cm. High-performance liquid chromatography (HPLC) profile of the proteins in WPI was analyzed on a size exclusion column (0.75 × 30 cm) (LKB Phamacia, Inc., Piscataway, NJ) using 20 mM phosphate buffer, pH 7.0, as the eluent. The elution profile, shown in Figure 1, was used as a reference to check uniformity in the protein composition of various batches of WPI. In addition, the pH–solubility profile of WPI in the range pH 2–9 was also used as a fingerprint to detect any protein denaturation in the batches of WPI received. Only those samples that showed similar HPLC profiles and pH–solubility profiles were used in the present study.

**Heat Treatment.** On the basis of preliminary experiments, two different heating conditions were chosen to denature WPI: In the first case, 40-mL aliquots of 9% WPI in water (pH 7.0) were heated at 90 °C for 5, 20, 40, 60, and 90 min. At the end of the heating period, the protein solutions were immediately diluted to 5% by rapid mixing with cold water; the solutions were immediately cooled under running cold tap water. The

* Author to whom correspondence should be addressed.

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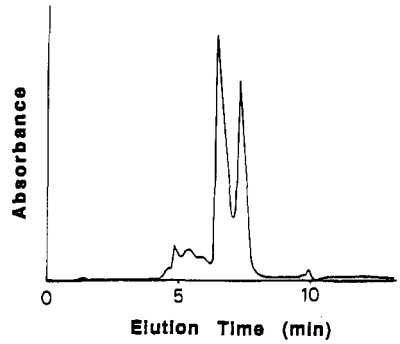


**Figure 1.** High-performance liquid chromatography profile of whey protein isolate. Water at pH 7 was used as the eluent.

resultant solutions under these conditions did not contain any visible precipitate and were translucent in appearance. These solutions were used immediately for foaming experiments. In the second case, 5% WPI solutions were heated at 70 °C for 1, 5, 10, and 20 min. The heated solutions were immediately cooled under running cold tap water and used as such for foaming studies.

**Electrophoresis.** Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of native and heated WPI was performed as described by Laemmli (1970) using 5–20% linear gradient slab gels. The molecular weight marker proteins phosphorylase *b* (94 000), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), soybean trypsin inhibitor (20 100), and α-lactalbumin (14 200) were from Pharmacia (Piscataway, NJ). The low molecular weight marker kit containing myoglobin peptide fragments was from Sigma Chemical Co., St. Louis, MO.

**Solubility and Surface Hydrophobicity Measurements.** The pH–solubility profiles of native and heated WPI were determined by measuring the turbidity of a 0.1% protein solution at 500 nm at various pHs as described by Kitabatake et al. (1985). The surface hydrophobicity of native and heated WPI was determined according to the procedure of Kato and Nakai (1980) using *cis*-parinaric acid as the fluorescent probe. The fluorescence measurements were made using a Perkin-Elmer Model LS-5 luminescence spectrophotometer.

**Secondary Structure Measurement.** Far-UV circular dichroism (CD) measurements were made in a modified and computerized Cary Model 60 spectropolarimeter (On-Line Instrument Systems, Jefferson, GA). A 0.1-cm path length quartz cell and a protein concentration of about 0.02% were used. Ten scans of each sample were averaged, and the mean residue ellipticity [θ] values, expressed as deg·cm$^2$·dmol$^{-1}$, were calculated using a value of 115 for the mean residue molecular weight. The CD spectra were corrected for the water baseline. The secondary structure contents were estimated from the CD spectra using a computer program based on the method of Chang et al. (1978).

**Viscosity and Surface Tension Measurements.** The viscosity of native and heated WPI solutions (5%) was determined using an Ostwald–Cannon–Fenske-type capillary viscometer immersed in a constant-temperature water bath maintained at 25 °C. The specific viscosity of protein solutions was calculated from the relation (Bradbury, 1970)

$$\eta_{sp} = (t_s - t_0)/t_0 \quad (1)$$

where $t_s$ and $t_0$ are the flow times for protein solution and water, respectively. Surface tension of protein solutions was determined by the Whilhelmy plate method using an electrobalance (Cahn Instruments Co., Cerritos, CA) as described previously (Xu and Damodaran, 1992).

**Kinetics of Foam Decay.** Recently, we have developed a simple and highly reproducible method to study foaming properties of proteins (Yu and Damodaran, 1991a,b). The method is based on physical principles that govern the equation of state of spherical foam particles. According to the Laplace equation, the equation of state of a stable spherical foam particle is given by

$$P_i - P_e = 4\gamma/r \quad (2)$$

where $P_i$ and $P_e$ are the pressures inside and outside the foam particle, $r$ is the radius, and $\gamma$ is the surface tension. If the foam is contained in a closed foam column, breakage of the foam would result in an increase in pressure inside the vessel. In other words, the rate of breakage of a foam inside a vessel can be monitored by measuring the rate of change of pressure inside the vessel. The pressure change inside the foam column can be transformed to changes in the interfacial area of the foam according to the relation (Nishioka and Ross, 1981)

$$A_t = (3V/2\gamma)(\Delta P_\infty - \Delta P_t) \quad (3)$$

where $A_t$ is the interfacial area of the foam at time $t$, $V$ is the total volume of the foam apparatus, and $\Delta P_t$ and $\Delta P_\infty$ are the net pressure changes at time $t$ and when the foam completely collapses at infinite time, respectively.

Using the above principle, a foam apparatus containing a single foam column with a differential pressure transducer attached at the top was constructed. The details of this apparatus and the procedures for measuring pressure change in the column as a function of time have been described previously (Yu and Damodaran, 1991a). The entire apparatus was housed in an incubator (VWR Scientific Inc., Model 1910) to precisely control the temperature. In a typical experiment, 20 mL of 5% WPI solution was placed in the solution chamber of the apparatus and preequilibrated at 25 ± 0.1 °C. Prepurified nitrogen gas was bubbled through the solution at a rate of 40 mL/min until the foam rose to a premarked point at the top of the column. The time required to raise the foam was recorded. All of the valves of the apparatus were closed, and the change in the pressure at the head space of the column was continuously recorded on a strip chart recorder. When the rate of change of pressure approached a plateau, an antifoaming agent stored in a bent side tube of the apparatus was dropped into the foam column to completely collapse the foam. The final pressure change ($\Delta P_\infty$) was recorded. Each experiment was done at least in triplicate.

A dimensionless fractional interfacial area at any given time during foam decay was calculated by using the relation (Yu and Damodaran, 1991a)

$$A_t/A_0 = (\Delta P_\infty - \Delta P_t)/\Delta P_\infty \quad (4)$$

where $A_0$ is the initial interfacial area of the foam, which is given by (Yu and Damodaran, 1991a)

$$A_0 = 3V\Delta P_\infty/2\gamma \quad (5)$$

The decay of WPI foams was analyzed according to the biphasic first-order equation (Yu and Damodaran, 1991a)

$$A_t/A_0 = Q_g \exp(-k_g t) + Q_d \exp(-k_d t) \quad (6)$$

where $k_g$ and $k_d$ are first-order rate constants for decay due to gravitational liquid drainage and interbubble gas diffusion, respectively, and $Q_g$ and $Q_d$ are amplitude parameters of the two kinetic phases.

Since $A_0$ is related to the foamability of the protein, the foaming activity index (FAI) of the protein, defined as the interfacial area per unit volume of the foam, was obtained from the relation

$$\mathrm{FAI} = A_0/v \quad (7)$$

where $v$ is the total volume of foam at $t = 0$.

## RESULTS AND DISCUSSION

The extent of heat denaturation and insolubilization of proteins in distilled water depends on the protein concentration, heating temperature, and heating time. By selecting various time–temperature–concentration conditions, it is possible to alter the physicochemical properties of WPI to any desired level. We have selected two specific temperature–concentration conditions, i.e., 90 °C–9% protein concentration and 70 °C–5% protein concentration, for this study. The rationale for selecting these

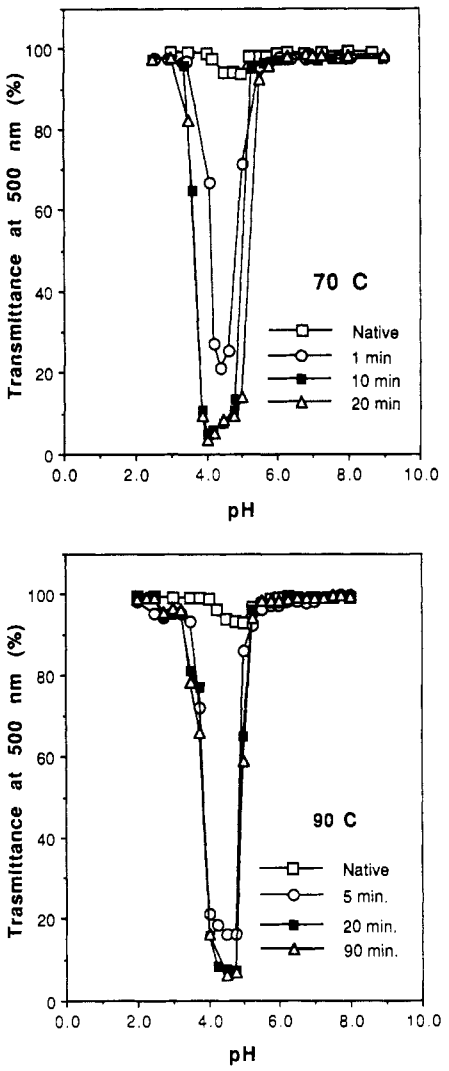


**Figure 2.** pH–solubility profile of WPI. (A, top) 5% WPI in 0.02 M NaCl at pH 7.0 was heated at 70 °C for various times; the heated solutions were diluted with 0.02 M NaCl to 0.1%, and the transmittance at 500 nm of the solutions was measured at various pHs. The pH was adjusted by adding 2 M NaOH or HCl. (B, bottom) Conditions were similar to those in (A) except that 9% WPI was heated at 90 °C for various times; the solutions were immediately diluted to 5% with 0.02 M NaCl and then diluted to 0.1% for turbidity measurement at various pHs.

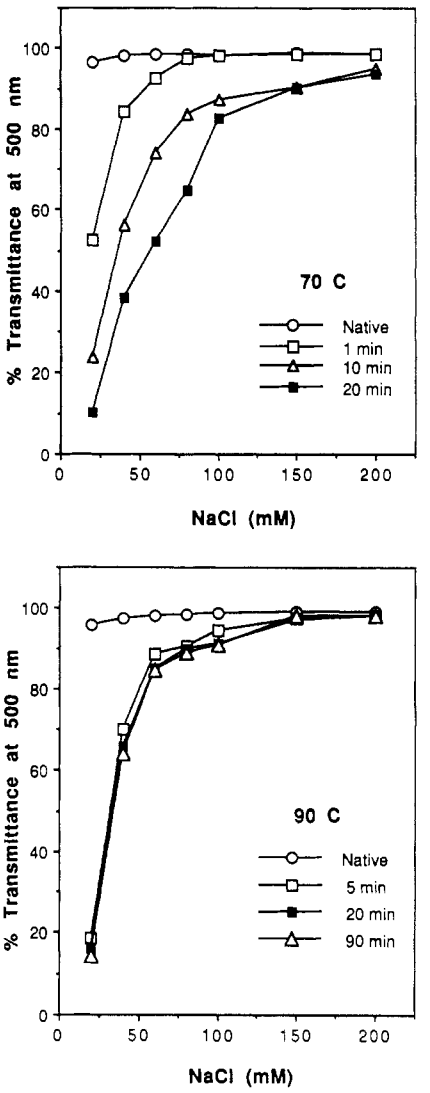


**Figure 3.** Solubility of WPI at pH 4.6 as a function of NaCl concentration. The protein concentration was 0.1%. (A, top) WPI (5%) heated at 70 °C; (B, bottom) WPI (9%) heated at 90 °C.

conditions was as follows: When a dilute WPI solution (<5%) is heated above the denaturation temperature of β-lactoglobulin, i.e., at 90 °C, the denatured whey proteins generally precipitate out of solution. On the other hand, when the concentration is above 10%, heating at 90 °C followed by cooling causes gelation of WPI. However, when a 9% WPI solution, which is just below the critical concentration needed for WPI gelation, is heated at 90 °C for up to 90 min, neither precipitation nor gelation of WPI occurs. The heated and cooled solution remains translucent. This is also the case when a 5% WPI solution is heated at 70 °C for up to 90 min. Since insoluble proteins cannot be formed, the above temperature–concentration conditions, which ensured protein in the soluble state, were chosen to alter the physicochemical properties of WPI.

**pH–Solubility Profile.** The native WPI used in these studies exhibited excellent solubility in the pH range 2–9 (Figure 2). The fact that no precipitation of α-lactalbumin and β-lactoglobulin occurred at their respective isoelectric pHs clearly indicated that the water-accessible surfaces of these proteins in their native state were highly hydrophilic, which promoted protein–solvent interactions instead of protein–protein (hydrophobic) interactions even at their isoelectric pH. However, the WPI samples heated at 70 and 90 °C for various times exhibited typical pH–solubility profiles with maximum solubility at pH 3 and above pH 6.0 and minimum solubility at about pH 4.5 (Figure 2). This loss of solubility indicated that the heat-induced exposure of previously buried hydrophobic groups enhanced the hydrophobic character of the protein surface that came in contact with the surrounding solvent, resulting in enhanced protein–protein interactions near the isoelectric pH of the major whey proteins.

The pH–solubility profiles of samples heated at 70 °C for 10 and 20 min and of samples heated at 90 °C for 5, 20, and 90 min were similar, implying that the extents of exposure of hydrophobic surfaces under these heating conditions were similar. To confirm whether this indeed was the case, the effect of NaCl on the solubility of these heated samples at pH 4.6 was studied (Figure 3). Because the extent of salting-in of proteins at the isoelectric region is a function of the hydrophobic character of the protein surface (Joly, 1965; Melander and Horvath, 1977), any differences in the salt concentration *vs* solubility profiles at pH 4.6 should be attributable to differences in the surface hydrophobic character of heat-denatured proteins. The data in Figure 3A show that the effectiveness of NaCl

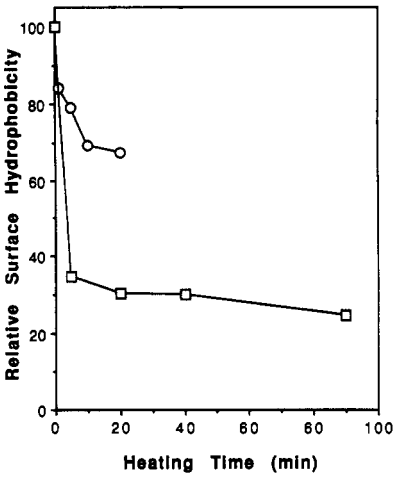


**Figure 4.** Changes in surface hydrophobicity of WPI as a function of heating time at 70 °C, 5% protein (○), and at 90 °C, 9% protein (□), conditions.

in solubilizing WPI heated at 70 °C decreased as the heating time was increased. This clearly indicates that the hydrophobic character of the protein surface was enhanced as the heating time at 70 °C was increased. In contrast, the 9% WPI solutions heated at 90 °C for 5, 20, and 90 min exhibited a greater salting-in behavior than the samples heated at 70 °C (Figure 3B), indicating that the surface characteristics of the samples heated at 90 °C were less hydrophobic than those heated at 70 °C. Apart from heating temperature, the major difference between these two heating conditions is the initial WPI concentration during heat treatment. The higher protein concentration (9%) used in the 90 °C heated samples apparently seems to protect the protein from extensive unfolding.

**Surface Hydrophobicity.** The changes in the relative surface hydrophobicity of WPI as a function of heating time at 70 and 90 °C are shown in Figure 4. The relative surface hydrophobicity, as measured by the *cis*-parinaric acid binding method (Kato and Nakai, 1980), decreased with heating time at both temperatures. However, the extent of decrease was much higher at 90 °C than at 70 °C. Voutsinas et al. (1983) reported that bovine serum albumin and β-lactoglobulin exhibited relatively higher surface hydrophobicity in the native state than in the heat-denatured state. The present results are in accord with those findings. It should be pointed out, however, that whereas the solubility experiments (Figures 2 and 3) clearly indicate that there was an increase in the hydrophobic character of the protein surface upon heating at both 70 and 90 °C, the surface hydrophobicity as measured by *cis*-parinaric acid binding suggests the opposite effect. This contradiction can be resolved only by reconciling that these two techniques probe two different aspects of the protein surface. It has been shown that binding of fluorescent probes such as *cis*-parinaric acid and 8-anilino-1-naphthalenesulfonic acid (ANS) to proteins occurs only at well-defined hydrophobic cavities formed by grouping of nonpolar residues on the protein surface (Damodaran, 1989). These cavities are not accessible to water but are accessible to apolar ligands. In this regard, these cavities do not contribute to the solubility characteristics of the protein. Individual hydrophobic residues randomly exposed at the protein surface do not have the ability to act as strong binding sites for fluorescent probes. It is known that β-lactoglobulin has a hydrophobic cavity which acts as the retinol binding site. This cavity may also act as the binding site for fluorescent probes. If the hydrophobic

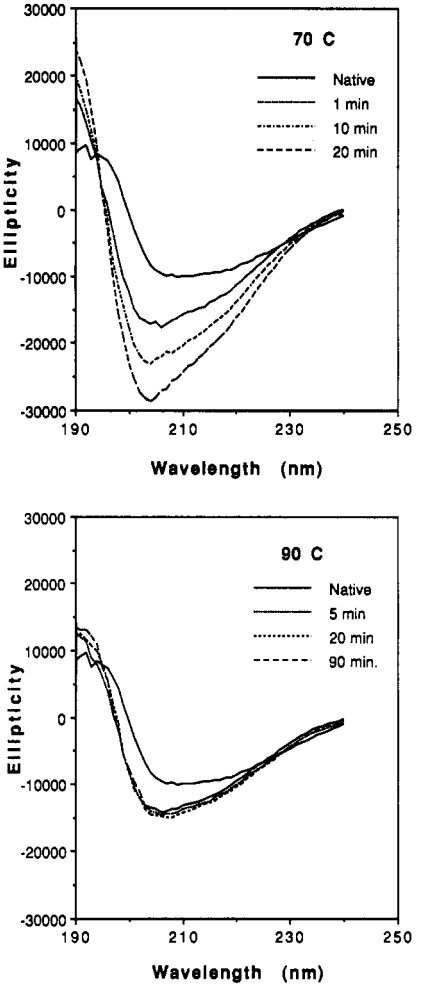


**Figure 5.** Far-UV CD spectra of WPI. (A, top) WPI heated at 70 °C; (B, bottom) WPI heated at 90 °C.

cavity is dissociated upon heating, and the constituent hydrophobic residues are individually distributed randomly on the protein surface, then no significant binding of the fluorescent probe to the protein would occur. On the other hand, the distribution of these hydrophobic residues on that part of the protein surface that comes into contact with solvent water would greatly enhance the hydrophobic character of the protein surface and would significantly affect the pH–solubility and salt-solubility profiles of the protein. In essence, the data in Figures 2 and 3, and in Figure 4, indicate that although the surface hydrophobic cavities of whey proteins are destroyed during heating, the hydrophobic character of the protein surface is actually enhanced.

**Conformational Changes.** The far-UV CD spectra of native and heat-denatured WPI are shown in Figure 5. The native WPI showed a broad negative peak in the 207–215-nm region. When heated at 70 °C (5%), the negative ellipticity of WPI increased with heating time and the wavelength of the negative peak shifted more toward 200 nm, indicating transconformation of α-helix and β-sheet structures to aperiodic structure. On the other hand, the net increase in the negative ellipticity of WPI samples heated at 90 °C (9%) was lower than the changes at 70 °C. In addition, major changes in the CD spectrum occurred within 5 min at 90 °C, and there were no further changes in the CD spectra when heated beyond 5 min (Figure 5). The secondary structure analysis of the CD spectra revealed that the native WPI contained an average of 20.5% α-helix, 42.5% β-sheet, 1.5% β-turns, and 34.5% aperiodic structure (Figure 6). Upon heating at 70 °C,

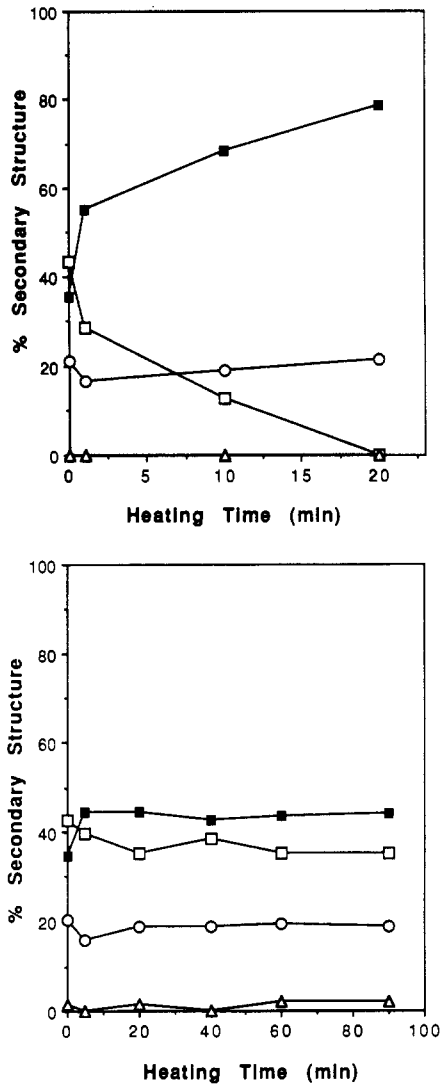


**Figure 6.** Changes in secondary structure content of WPI as a function of time at 70 (A, top) and 90 °C (B, bottom) heating conditions. (○) α-Helix; (□) β-sheet; (△) β-turns; (■) aperiodic structures.

the aperiodic structure increased and the β-sheet structure decreased with heating time. There were no significant changes in α-helix and β-turns. The β-sheet structure of WPI disappeared completely within 20 min, indicating that the increase in aperiodic structure was solely at the expense of the β-sheet structure. In contrast, the WPI samples that were heated at 90 °C did not exhibit major changes in the secondary structure content (Figure 6). The β-sheet content progressively decreased from about 42.5% in the native state to only about 35% after 90 min. The aperiodic structure increased from about 34.5% to about 44%. The α-helix and β-turn contents did not change. It is probable, although unlikely, that the minimal change in β-sheet content at 90 °C heating conditions could be due to an increase in one of the whey proteins and a decrease in another whey protein. Nonetheless, comparison of gross structural changes in WPI upon heating at 70 (5%) and 90 °C (9%) clearly indicates that major unfolding of whey proteins occurred only at the 70 °C heating conditions but not at the 90 °C heating conditions. The major factor that has affected protein unfolding seems to be the difference in the protein concentration. The higher protein concentration used at 90 °C heating conditions apparently had a protective effect on thermal unfolding of the proteins. The fact that the hydrophobic character of the samples heated at 70 °C was greater than

**Table 1. Effect of Heat on Specific Viscosity of WPI Solutions (5%, 25 °C, pH 7.0)**

| | specific viscosity[a] at heating condition (temp, concn) | |
|---|---|---|
| heating time (min) | 70 °C, 5% | 90 °C, 9% |
| 0 | 0.21 ± 0.01 | 0.21 ± 0.01 |
| 1 | 0.28 ± 0.01 | |
| 5 | 0.28 ± 0.01 | 1.87 ± 0.00 |
| 10 | 0.32 ± 0.01 | |
| 20 | 0.43 ± 0.01 | 2.25 ± 0.01 |
| 40 | | 2.33 ± 0.01 |
| 60 | | 2.38 ± 0.01 |
| 90 | | 2.30 ± 0.01 |

[a] Average of triplicate measurements ± standard error.

that of those heated at 90 °C, as evidenced from the salting-in profiles (Figure 3), concurs well with the changes in the secondary structures.

**Hydrodynamic Changes.** The specific viscosity of a 5% native WPI solution was about 0.208 at 25 °C. Upon heat treatment at 70 °C, the specific viscosity increased with heating time and attained a value of 0.43 after 20 min (Table 1). In the case of WPI samples (9%) heated at 90 °C (diluted to 5% final concentration), the increase in specific viscosity was much higher than that of the samples heated at 70 °C (Table 1). For example, at 20-min heating time the specific viscosity of 90 °C heated sample was about 2.25 compared to 0.43 for the 70 °C heated sample (Table 1). The major changes in the specific viscosity of 90 °C heated samples occurred within 20 min heating time; no significant increase in specific viscosity was observed after 20 min.

The two most important factors that affect viscosity of protein solutions are the hydrodynamic shape and the size of the protein molecules, which follow the relationship

$$\eta_{sp} = \beta C(\upsilon_2 + \delta_1\upsilon_1) \quad (8)$$

where $\beta$ is the shape factor, $C$ is the concentration, $\upsilon_2$ and $\upsilon_1$ are the specific volumes of the protein and the solvent, respectively, and $\delta_1$ is the weight of solvent bound per unit weight of the protein. Thus, the rapid increase in the specific viscosity of the WPI sample heated at 90 °C for 5 min must be due to remarkable increase in the hydrodynamic size and shape of whey proteins. It should be pointed out, however, that this remarkable increase in the specific viscosity cannot arise only from unfolding of the secondary and tertiary structures of the proteins, because such structural changes were insignificant in the case of the 90 °C, 9% heated samples compared to those of the 70 °C, 5% heated samples (Figure 6B). Alternatively, therefore, the higher viscosity of the 90 °C, 9% heated samples must be related to formation of soluble polymers, possibly via sulfhydryl–disulfide interchange reactions.

To elucidate whether the dramatic increase in the specific viscosity of WPI heated at 90 °C, 9% conditions was due to polymerization via sulfhydryl–disulfide interchange reaction, the SDS–PAGE patterns of the heat-treated samples were analyzed under reducing and nonreducing conditions (Figure 7). Electrophoresis under nonreducing conditions revealed that the majority of proteins in WPI heated at 90 °C were unable to enter the 4% stacking gel (Figure 7A), indicating that the molecular weights of these polymer species were more than 1 million (Utsumi et al., 1984). The concentrations of monomeric α-lactalbumin and β-lactoglobulin in these samples were very low, indicating that these proteins were polymerized to high molecular weight soluble polymers within 5 min of heating at 90 °C. When the samples were electro-

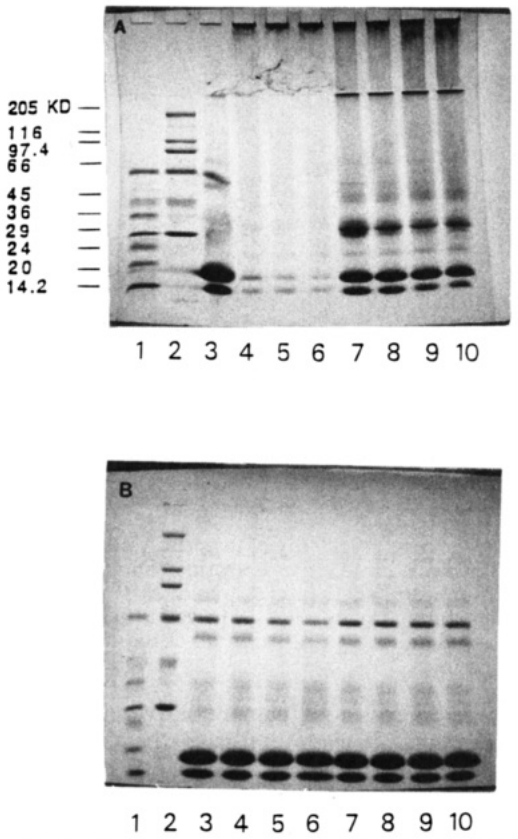


**Figure 7.** Heat-induced polymerization of WPI: (A) SDS–PAGE patterns of heated WPI. Electrophoresis was run under nonreducing conditions. Wells 1 and 2 are molecular weight markers; well 3 is native WPI. Wells 4, 5, and 6 are 9% WPI heated at 90 °C for 5, 20, and 90 min, respectively; wells 7, 8, 9, and 10 are 5% WPI heated at 70 °C for 1, 5, 10, and 20 min, respectively. (B) Same as in (A), but electrophoresis was run after the samples were reduced with with 0.5 M β-mercaptoethanol.

phoresed after reduction in the presence of dithiothreitol (DTT), the polymers that were unable to enter the stacking and separating gels disappeared with concomitant appearance of the monomeric α-lactalbumin and β-lactoglobulin bands (Figure 7B), indicating that the polymers were formed via disulfide–sulfhydryl interchange reactions. In the case of WPI samples heated at 70 °C conditions, the electrophoretic patterns of nonreduced samples showed polymeric bands at the top of the stacking and separating gels as well as monomeric bands corresponding to α-lactabumin and β-lactoglobulin in the separating gel (Figure 7A). The intensities of the monomeric α-lactalbumin and β-lactoglobulin bands gradually decreased with increase of heating time. In addition to the polymeric and monomeric protein bands, a protein band corresponding to a molecular weight of about 31 000, which was not present in the unheated WPI, appeared in the 70 °C heated samples. Since the molecular weight of this protein band is the sum of the molecular weights of monomeric α-lactalbumin (12 400) and monomeric β-lactoglobulin (18 600), this band might be an α–β dimer formed via sulfhydryl–disulfide interchange reaction during heat treatment. The absence of this band in the 90 °C heated sample implies that the α-β dimer was further polymerized via sulfhydryl–disulfide interchange reactions to higher molecular weight polymers that could not enter the stacking gel. The data indicate that under 70 °C and 5% heating conditions only a fraction of α-lactalbumin and β-lactoglobulin is polymerized to high molecular weight soluble polymers, and the extent of polymerization is time dependent. When the 70 °C heated samples were electrophoresed after reduction with DTT, the high molecular weight polymers disappeared, confirming that these polymers were formed via disulfide–sulfhydryl interchange reactions between α-lactalbumin and β-lactoglobulin (Figure 7B).

**Table 2. Effect of Heat Treatment on the Initial Interfacial Area, $A_0$ (cm²), of WPI Foams[a]**

| | heating conditions (temp, concn) | | | |
|---|---|---|---|---|
| | 70 °C, 5% | | 90 °C, 9% | |
| heating time (min) | $A_0 \times 10^{-3}$ (cm²) | FAI (cm⁻¹) | $A_0 \times 10^{-3}$ (cm²) | FAI (cm⁻¹) |
| 0 | 23.4 ± 0.9 | 349 ± 13 | 23.4 ± 0.9 | 349 ± 13 |
| 1 | 27.2 ± 0.4 | 406 ± 6 | | |
| 5 | 24.6 ± 0.4 | 367 ± 6 | 20.6 ± 0.7 | 307 ± 10 |
| 10 | 23.0 ± 1.0 | 343 ± 15 | | |
| 20 | 21.0 ± 0.8 | 313 ± 12 | 20.0 ± 0.8 | 298 ± 12 |
| 90 | | | 19.3 ± 1.3 | 288 ± 19 |

[a] Average of triplicate measurements ± standard error.

The data on the changes in physicochemical properties of WPI heated under the two chosen conditions clearly indicate the following: When heated at 70 °C and 5% conditions, the whey proteins underwent rapid conformational changes within a short heating time. The aperiodic structure content of the proteins increased primarily at the cost of β-sheet structure. The hydrophobic character of the protein surface was enhanced, as evidenced by changes in the pH–solubility profile and the solubility profile in NaCl solutions at pH 4.6. However, the surface hydrophobicity decreased. In contrast, when heated at 90 °C and 9% conditions, whey proteins did not undergo extensive conformational change as evidenced by insignificant changes in the secondary structure parameters. Although the pH–solubility profile showed minimum solubility at about pH 4.6, the data on the solubility profile in NaCl solutions at pH 4.6 suggested that the hydrophobic character of the protein surface was not greatly enhanced. The specific viscosity data and the gel electrophoretic patterns indicated that the samples heated at 90 °C, 9% conditions underwent extensive polymerization via sulfhydryl–disulfide interchange reactions, whereas such polymerization was minimal in the case of 70 °C, 5% heated samples. To elucidate the influence of these heat-induced physicochemical changes on the surface active properties of WPI, the foaming properties were studied.

**Foamability of Heat-Denatured WPI.** In preliminary studies it was observed that at 1% protein concentration native WPI could not form a reasonable volume of foam when sparged with nitrogen. However, at 5% protein concentration it was possible to generate the volume of foam required for monitoring the kinetics of foam decay using the foam apparatus used in this study.

The initial interfacial area ($A_0$) and the FAI of WPI foams, calculated from $\Delta P_\infty$ by using eqs 5 and 7, respectively, are presented in Table 2. In all of these experiments the total volume of foam generated was 67 mL. In the case of 70 °C heated samples, both $A_0$ and FAI exhibited an increase when heated for 1 min and then decreased with heating time. The total initial interfacial area of the foam of 20-min-heated sample was significantly lower than that of the native WPI foam. The data indicate that the WPI sample heated at 70 °C for 1 min possessed the molecular properties required to form a denser foam with smaller average bubble size than either the native or WPI heated for longer time. In contrast, $A_0$ and FAI of the WPI samples heated at 90 °C progressively decrease with heating time (Table 2), indicating an increase in the average bubble size with heating time. It should be pointed

**Table 3. Effect of Heat Treatment on the Time Required to Create 67 mL of WPI Foam[a]**

| | foaming time (s) at heating condition (temp, concn) | |
|---|---|---|
| heating time (min) | 70 °C, 5% | 90 °C, 9% |
| 0 | 106.6 ± 2.4 | 106.6 ± 2.4 |
| 1 | 86.4 ± 0.8 | |
| 5 | 85.1 ± 0.2 | 177.8 ± 8.1 |
| 10 | 122.9 ± 3.1 | |
| 20 | 133.0 ± 6.0 | 188.2 ± 1.8 |
| 40 | | 167.6 ± 6.6 |
| 90 | | 174.5 ± 6.4 |

[a] Average of duplicate measurements ± standard error.

out, however, that, irrespective of the heating conditions, prolonged heating of WPI at either 70 or 90 °C caused formation of foams with larger bubbles and consequently a decrease in FAI. In fact, the data in Table 2 suggest that as the heating time was increased the FAI apparently approached a limiting value of about 288 $cm^2/mL$ at both the 70 and 90 °C heating conditions.

The effect of heating time on the time required to form 67 mL of foam at a nitrogen gas flow rate of 40 mL/min is presented in Table 3. The foaming time significantly dropped in the case of WPI heated at 70 °C for 1 and 5 min and then increased for samples heated for longer times. On the other hand, in the case of 90 °C heated samples, only an increase in foaming time was observed; the major increase in foaming time occurred within 5 min, and further heating up to 90 min did not show any significant additional increase in foaming time (Table 3).

The observed effects of time–temperature–concentration treatments on foamability and foaming activity index of WPI clearly indicate that the extent of heat-induced changes in the physicochemical properties of WPI significantly affects its foamability. The foamability of proteins is fundamentally related to their film-forming ability at the air–water interface (Mita et al., 1977, 1978). In general, proteins that rapidly adsorb at the air–water interface and readily undergo unfolding and molecular rearrangement at the interface often exhibit better foamability than those proteins that adsorb slowly and resist unfolding at the interface. The fact that the WPI heated at 70 °C for 1 min exhibited better foamability than either the unheated WPI or any of the other heat-treated samples clearly indicates that the structural changes in proteins under this heating condition imparted optimum molecular characteristics for better foamability. In terms of secondary structure content this optimum conformation is related to about 28.5% β-sheet, 16.5% α-helix, and 55% aperiodic structures. It should be emphasized, however, that it is the changes in the tertiary structure, and the consequent changes in the physicochemical aspects of the protein surface and conformational stability of the protein at the interface, that are more critical than changes in the secondary structure *per se*. It is generally believed that highly flexible random coil proteins would exhibit better foamability than rigid and highly ordered proteins (Graham and Phillips, 1976, 1979). However, the results presented here apparently contradict this view, because if this indeed is the case, then the WPI sample heated at 70 °C for 20 min, which contained 78.5% random coil, 21.5% α-helix, and no β-sheet, should have exhibited better foamability than any of the other heat-denatured samples. The results strongly suggest that for a protein to exhibit better foamability, the protein should contain an optimum degree of folded conformation.

**Stability of WPI Foams.** The kinetics of surface area decay of foams of native and 70 °C heated WPI are shown

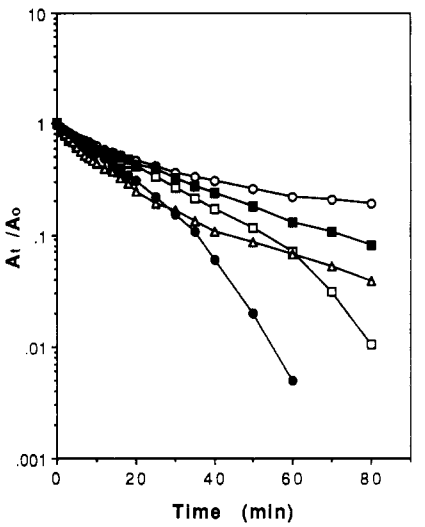


**Figure 8.** Surface area decay of foams of WPI samples heated at 70 °C and 5% protein concentration conditions for various times: □, unheated; ○, 1 min; ■, 5 min; △, 10 min; ●, 20 min. Foams were formed using 5% WPI in water (pH 6.8, 25 °C). The average standard error of $A_t/A_0$ for all of the curves was $5.12 \times 10^{-3}$.

in Figure 8. The decay of foams of native and 70 °C heated WPI samples followed nonlinear first-order kinetics, apparently involving at least two kinetic phases (Yu and Damodaran, 1991a). It has been suggested that breakage of protein-stabilized foams involves two microscopic processes (Yu and Damodaran, 1991a). These two processes are gravitational drainage of liquid from the lamella and disproportionation of gas bubbles due to interbubble gas diffusion. Since these two microscopic events are fundamentally involved in foam decay, it is logical to expect that these two processes must occur irrespective of whether the kinetics exhibit biphasic or monophasic first order. In other words, the kinetics of foam decay should conform to eq 6. If a foam exhibits an apparent monophasic first-order kinetics, then the rate constants $k_g$ and $k_d$ should be equal in magnitude, and these two processes must occur simultaneously. In addition, since the magnitude of foam decay by interbubble gas diffusion is significant only below a critical film thickness, the first kinetic phase in a biphasic first-order decay should be predominantly attributable to the gravitational drainage process (Yu and Damodaran, 1991a).

Heating of WPI for 1 min at 70 °C caused an increase in foam stability; prolonged heating, however, progressively decreased the foam stability (Figure 8). The foams of 1-, 5-, and 10-min-heated WPI samples exhibited a concave-type decay curve, whereas the native and 20-min-heated samples exhibited a convex-type decay curve. The concave shape indicates that the rate of decay due to gravitational decay was greater than that due to interbubble gas diffusion. In other words, in the case of 1-, 5-, and 10-min-heated samples the rate of interbubble gas diffusion appears to be the rate-limiting step in the decay of these foams. On the other hand, the convex-type decay curves of native and 20-min-heated samples indicate that the gravitational liquid drainage, and not the interbubble gas diffusion, is rate limiting in these foams. The effect of heating time at 70 °C on the rate constants $k_g$ and $k_d$ of WPI foams is presented in Figure 9. The magnitude of $k_g$ and $k_d$ was the same for the native WPI foam. $k_g$ slightly increased as a function of heating time. On the other hand, $k_d$ exhibited an initial decrease in samples heated for up to 10 min and dramatically increased for the 20-min-heated sample.

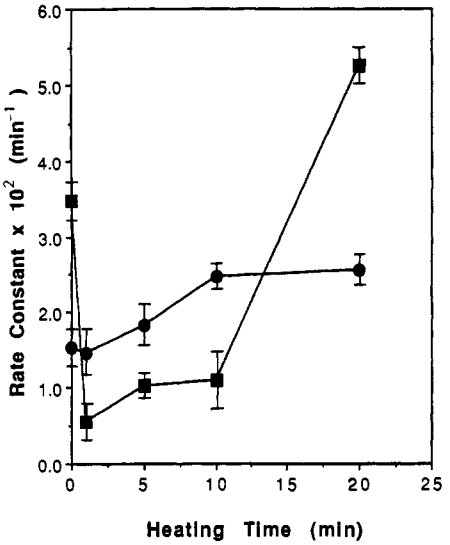


**Figure 9.** Effect of heating time at 70 °C (5% protein concentration) on the gravitational (●) and gas diffusional (■) rate constants of decay of WPI foams (5%, pH 6.8) at 25 °C. The bars represent standard errors.

The transformation from convex- to concave-type and from concave- to convex-type biphasic first-order decay of WPI foams as a function of heating time at 70 °C reflects fundamental changes in physical properties of the protein film. Heating of WPI at 70 °C for up to 20 min caused only a slight increase in the gravitational drainage rate (Figure 9). Since gravitational drainage is influenced by the viscoelasticity of protein films (Yu and Damodaran, 1991a), the physicochemical changes in WPI under these heating conditions apparently did not cause a dramatic decrease in the viscoelastic properties of the protein films at the air–water interface. The initial decrease in $k_d$ in fact suggests that the partially unfolded protein molecules were able to form a viscoelastic film with decreased gas permeability and increased mechanical strength. However, in the case of 20-min-heated sample the $k_4$ increased dramatically, indicating that in the highly denatured state whey proteins were unable to form a cohesive film with low gas permeability.

In contrast to the foaming behavior of 70 °C heated WPI samples, the foams of 90 °C heated WPI samples exhibited a dramatic decrease in their stability compared to that of the native WPI (Figures 10 and 11). In addition, the decay curves were essentially monophasic first order, indicating that both gravitational drainage and gas diffusion processes occurred simultaneously and contributed to rapid decay of the foams. It is not readily apparent what critical physicochemical changes in WPI during heating for various times at 70 and 90 °C are responsible for the observed variations in the foaming properties. Specifically, it is not clear what conformational and hydrodynamic changes in the proteins confer high foam stability for the sample heated for 1 min at 70 °C and poor foam stability for the other 70 and 90 °C heated samples. In qualitative terms, it appears that when the secondary structure content of WPI is about 28.5% β-sheet, 16.5% α-helix, and the remainder aperiodic structure, both foamability and foam stability are improved. However, what role, if any, the extent of polymerization in WPI heated for 1 min at 70 °C has on the foaming properties is not readily apparent. It should be pointed out that all samples that were heated at 90 °C were almost completely in the polymerized state, with molecular weight over a million. On the other hand, all of the samples that were heated at 70 °C contained both monomeric and polymeric

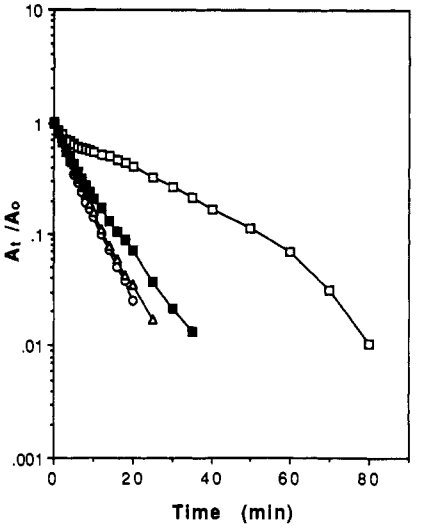


**Figure 10.** Surface area decay of foams of WPI samples heated at 90 °C and 9% protein concentration conditions for various times: □, unheated; ○, 5 min; △, 20 min; ■, 90 min. Foams were formed using 5% WPI in water (pH 6.8, 25 °C). The average standard error of $A_t/A_0$ for all of the curves was $4.92 \times 10^{-2}$.

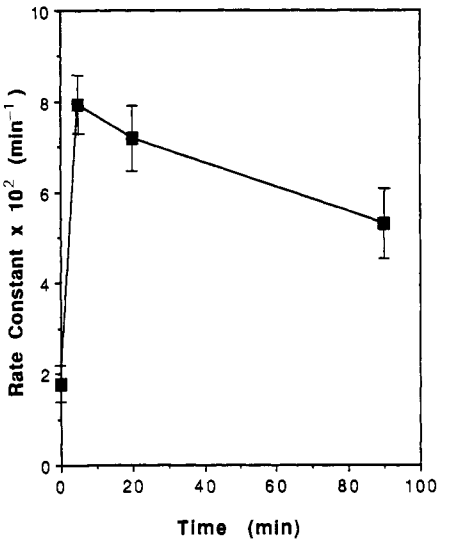


**Figure 11.** Effect of heating time at 90 °C (9% protein) on the rate constant of decay of WPI foams (5%, pH 6.8) at 25 °C. The bars represent standard errors.

protein species, as well as an α–β dimer. However, among these samples, only the sample that was heated for 1 min at 70 °C exhibited improved foaming properties. This tentatively suggests that, in addition to the secondary structure requirements, the extent of polymerization, i.e., the ratio of monomeric to polymeric species in the heated samples, might also play a role in improving the foaming properties of WPI.

To elucidate whether the ratio of monomeric to polymeric species affects the foaming properties of WPI, experiments on the foaming properties of mixtures of native WPI and WPI heated at 90 °C for 20 min were conducted. The data in Figure 12 show that when the heat-denatured WPI was mixed with native WPI at various ratios (w/w), the foam stability increased dramatically at a native to heated WPI ratio of 40:60; further addition of native WPI above 40% level to the heated WPI caused a progressive decrease in foam stability. The foam decay curve for the 40:60 mixture was very similar to that of WPI heated for 1 min at 70 °C (Figure 8), suggesting that the monomer to polymer ratio in this heated sample also might be close to 40:60. The effect of the ratio of native

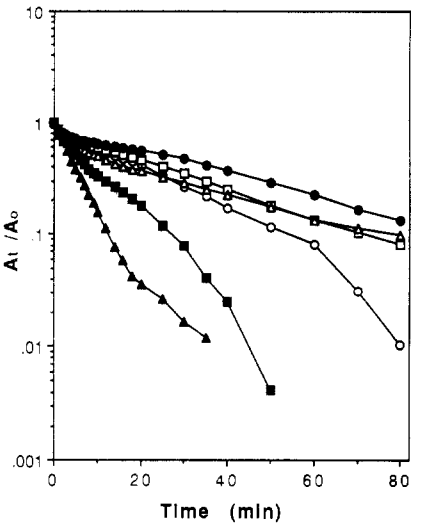


**Figure 12.** Effect of mixing native WPI with 90 °C (9%, 20 min) heated WPI at various ratios on the surface area decay of WPI foams. The final WPI concentration was 5%. Fraction of native WPI in the protein solution: ▲, 0%; ■, 20%; ●, 40%; △, 60%; □, 80%; ○, 100%. The average standard error of $A_t/A_0$ for all of the curves was $6.16 \times 10^{-3}$.

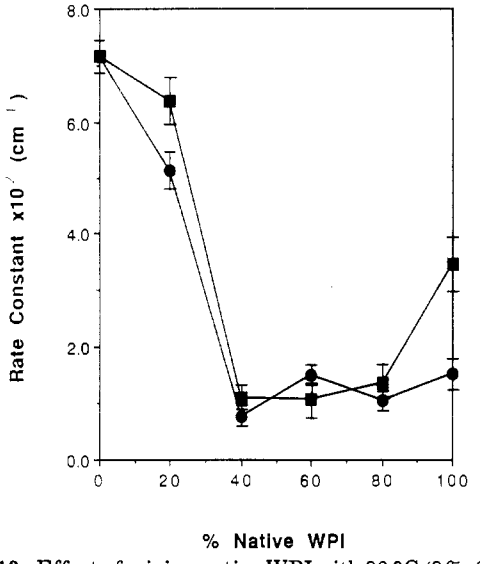


**Figure 13.** Effect of mixing native WPI with 90 °C (9%, 20 min) heated WPI at various ratios on the gravitational (●) and gas diffusional (■) rate constants of decay of WPI foams. The bars represent standard errors.

to heated WPI on the decay rate constant $k_g$ and $k_d$ is shown in Figure 13. Both rate constants decreased rapidly when up to 40% (w/w) native WPI was added to the 90 °C heated sample. Only a slight increase in the rate constants was observed with further addition of native WPI. The effect of native WPI addition on the initial interfacial area ($A_0$) of the heated WPI foam is shown in Figure 14. In contrast to the behavior of foam stability, $A_0$ was almost unchanged when up to 40% native WPI was added to the 90 °C heated WPI. However, when the native WPI content was increased to 60%, a dramatic increase in $A_0$ was observed (Figure 14). At higher native WPI incorporation, the $A_0$ decreased progressively and approached a value typical for 100% native WPI foam.

The data clearly indicate that the ratio of monomeric to polymeric proteins in heat-denatured WPI appears to be critical to its foaming properties. While the foam stability seems to be better when the ratio of monomer to polymer is 40:60, the foamability is better at 60:40 ratio. Although the fundamental reasons for these requirements

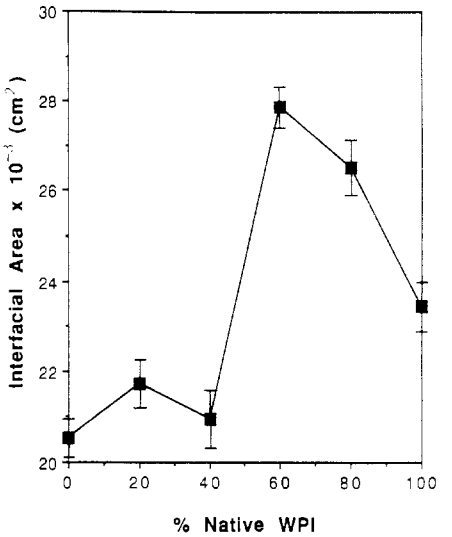


**Figure 14.** Effect of mixing native WPI with 90 °C (9%, 20 min) heated WPI at various ratios on the initial interfacial area of WPI foams. The bars represent standard errors.

are not clear, it appears that the monomeric species contribute to foam formation and the polymeric species contribute to foam stability. Since the diffusion coefficients of monomeric α-lactalbumin and β-lactoglobulin are higher than the polymeric species, they rapidly migrate, readily adsorb and unfold, and form a film at the air–water interface during bubbling. The protein films formed by monomeric proteins do not appear to have the required viscoelastic properties to stabilize the foam. However, when the late arriving polymeric species adsorb to the preformed film, they increase the viscoelastic properties of the film and thus stabilize the foam against gravitational drainage and interbubble gas diffusion. When only polymeric species are present in the protein solution, as in the case of WPI heated at 90 °C for 20 min, because of their low diffusion coefficient, they do not readily adsorb to the air–water interface as more and more interfacial area is being created during bubbling. The fact that it requires a longer time for the 90 °C heated WPI sample than for the native WPI to create the same volume of foam (Table 3) is essentially related to this phenomenon. In addition, once adsorbed at the interface, because of lack of conformational flexibility, the high molecular weight polymers do not readily undergo unfolding at the interface and rapidly decrease the interfacial tension; this results in decreased foam stability. It appears that in the case of WPI containing both monomeric and polymeric proteins, the monomers contribute to rapid lowering of interfacial tension (which enhances foamability) and the polymers contribute to film viscosity and elasticity (which enhance foam stability).

The results presented here clearly demonstrate that heat-induced changes in the physicochemical properties of WPI greatly influence its foaming properties. In addition to conformational changes and the changes in the hydrodynamic properties and hydrophobic character of the protein surface, the ratio of monomeric to polymeric species in heated WPI also greatly influences both foamability and stability of WPI foams. On the basis of the results presented here, it is clear that the foaming properties of WPI can be greatly improved through appropriate heat treatment and/or through mixing of native and heat-treated WPI.

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