Dynamic Interfacial Rheology as a Tool for the Characterization of Whey Protein Isolates Gelation at the Oil−Water Interface

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Heat-induced interfacial aggregation of a whey protein isolate (WPI), previously adsorbed at the oil−water interface, was studied by interfacial dynamic characteristics coupled with microscopic observation and image analysis of the drop after heat treatment. The experiments were carried out at temperatures ranging from 20 to 80 °C with different thermal regimes. During the heating period, competition exists between the effect of temperature on the film fluidity and the increase in mechanical properties associated with the interfacial gelation process. During the isothermal treatment, the surface dilational modulus, E, increases, and the phase angle, δ, decreases with time to a plateau value. The frequency dependence of E and δ is characteristic of viscoelastic films with increasing δ and decreasing E at lower frequencies. The effects of heat treatment depend on the conditions at which the gelation process takes place. Microscopic observation of gelled films gives complementary information on the effect of heat treatment on WPI adsorbed films.

Keywords: *Whey protein isolate (WPI); interfacial gelation; adsorbed protein; interfacial rheology; interfacial tension; oil−water interface; heat-treated protein; dynamic interfacial properties*

INTRODUCTION

Some important food colloids are protein particle gels formed by aggregation of proteinaceous or protein-coated colloidal particles. Gels are responsible for many functional properties, such as water holding, diffusion, and rheological properties, and thus the gel-forming properties of proteins have received great attention (Kinsella, 1984; Kinsella and Whitehead, 1989; Das and Kinsella, 1990; Boye et al., 1997; Oakenfull et al., 1997; Cayot and Lorient, 1997; Mulvihill and Donovan, 1987; Phillips et al., 1994; Ziegler and Foegeding, 1990). There are several gelation mechanisms for proteins. Gelation may be induced by heating, by addition of acid or salts, or by treatment with enzymes (Oakenfull et al., 1997; Ziegler and Foegeding, 1990).

Thermally induced gelation may be view as a two-stage sequential process (Ball and Jones, 1995; Clark et al., 1981; Ferry, 1948). The first step involves heat-induced conformational changes in a protein which may involve unfolding of some polypeptide segments followed by sequential protein−protein interactions (cross-linking) resulting in a progressive build up of a network structure when protein−protein interaction is limited. Noncovalent protein−protein interactions occur in the formation of gels. The number or type of noncovalent interactions vary with the protein, pH, heat treatment, and ions present. Cross-linking is essential for gel formation, which together with the solvent provides the fluidity, mechanical strength, viscoelasticity, and flow behavior of gels. Gelation transforms the material from a viscous liquid into a solidlike material which is quite elastic in its response to deformation. Thus, rheology is an important tool for observing physical characteristic changes during gelation, influenced by variables such as time, temperature, pH, protein concentration, etc. or a combination of these variables (Oakenfull et al., 1997; Ziegler and Foegeding, 1990).

Whey proteins possess useful gelling properties which are of interest for the food industry. The major protein in whey is β-lactoglobulin. It is a globular protein which contain 162 amino acid residues and has a molecular mass of approximately 18 300 Da. At room temperature β-lactoglobulin exists as a dimer in water at a pH between 5.5 and 7.5. The monomer units contain two intermolecular disulfide bonds and one thiol group (Swaisgood, 1982). On heating β-lactoglobulin solutions, several intramolecular and intermolecular changes take place. Raising the temperature to 30−50 °C induces the dissociation of the dimer into its monomer units, and at temperatures above approximately 60 °C the monomers undergo a denaturation step in which they unfold. The denaturation can be followed by an irreversible aggregation so that the whole process becomes irreversible (Ball and Jones, 1995; Clark et al., 1981; Phillips et al., 1994; Kinsella et al., 1994). Although many studies have been performed on the heat-induced denaturation and aggregation of β-lactoglobulin, the precise mechanism, or mechanisms, by which the reactions take place remains unclear. This is due in part to the fact that the changes that take place on heating β-lactoglobulin are influenced by many factors such as electrostatic and hydrophobic interactions, hydrogen bonding, and disulfide cross-linking (Mulvihill et al., 1990).

The aim of this article was to introduce surface dilational measurements as a very sensitive technique to investigate the viscoelastic properties of heat-set induced WPI protein gel at the oil−water interface. Heat-induced interfacial aggregation of globular proteins is not a well-known phenomenon, even though it possesses great technological importance (Dickinson,

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Table 1. Chemical Specification of Whey Protein Isolate (WPI)

	%
protein (N × 6.38)	92 ± 2
β-lactoglobulin	>95
α-lactoglobulin	<5
fat	<1
lactose	<0.2
moisture	5.5
nitrogen solubility index at pH 4.5 (1% solution, 16000*g*, 30 in)	>95

1992). The problem is connected with the fact that protein denaturation and aggregation could be associated with the adsorption and further heat-gelation in a complicated manner. As far as we know, in only a few papers the surface gelation of polymers by tensiometry (Kim et al., 1984) and that of protein by means of attenuated total reflection Fourier transformation infrared spectroscopy, AT-FITR, (Ball and Jones, 1995; Green et al., 1999) have been investigated. As to the interaction between protein-adsorbed molecules at the air−water (Rodríguez Niño et al., 1997a, b, 1998) and oil−water interface (Rodríguez Patino et al., 1999), insight can be expected from systematic studies on interfacial rheology coupled with optical methods applied to bubbles or drops.

MATERIALS AND METHODS

Chemicals. Whey protein isolate (WPI), a native whey protein with a very high content of β-lactoglobulin (Table 1) obtained by fractionation, was supplied by Danisco Ingredients (Denmark). The sample was stored below 0 °C, and all work was done without further purification. Samples for interfacial characteristics of WPI films were prepared using Milli-Q ultrapure water and were buffered at pH 5.0. Trisun oil (fatty acid composition, C16, 4%; C18, 4%; C18:1, 80%; C18:2, 9%; C18:3, traces; C20, 0.5%; and C22, 1%) supplied by Danisco Ingredients, was Florisil 60−100 mesh (Aldrich)-treated to remove any surface-active impurities. Analytical grade acetic acid and sodium acetate for buffered solutions were used as supplied by Sigma (>95%) without further purification. The absence of active surface contaminants in the aqueous buffered solutions was checked by interfacial tension measurements before sample preparation. No aqueous solutions with a surface tension other than that accepted in the literature (72−73 mN/m at 20 °C) were used.

Methods. For interfacial tension and surface dilational property measurements of adsorbed protein films at the oil−water interface an automatic drop tensiometer developed by Labordenne et al. (1994) was utilized. Figure 1 shows a diagram of the experimental setup. Depending on the density of the two fluids, the drop is either mounting (oil drop in water) or hanging (water drop in oil). After formation of the drop, its profile is digitized and analyzed through the CCD camera coupled to a video image profile digitizer board connected to a microcomputer. To retain permanent visual control, the drop image is continuously visualized on a video monitor. The drop profile is processed according to the fundamental Laplace equation in order to obtain the interfacial or surface tension

$$\frac{1}{x}\frac{\mathrm{d}}{\mathrm{d}x}(x \sin \Theta) = \frac{2}{b} - Cz \qquad (1)$$

where x and z are the Cartesian coordinates at any point of the drop profile, b is the radius of curvature of the drop apex, Θ is the angle of the tangent to the drop profile, and C is the capillary constant, $C = g\Delta\rho/\sigma$, where σ is the interfacial tension, $\Delta\rho$ is the difference between the densities of the two liquids, and g is the acceleration of gravity. The computer calculates three characteristic parameters of the drop, namely, the area, A, volume, V, and interfacial tension, σ. The average

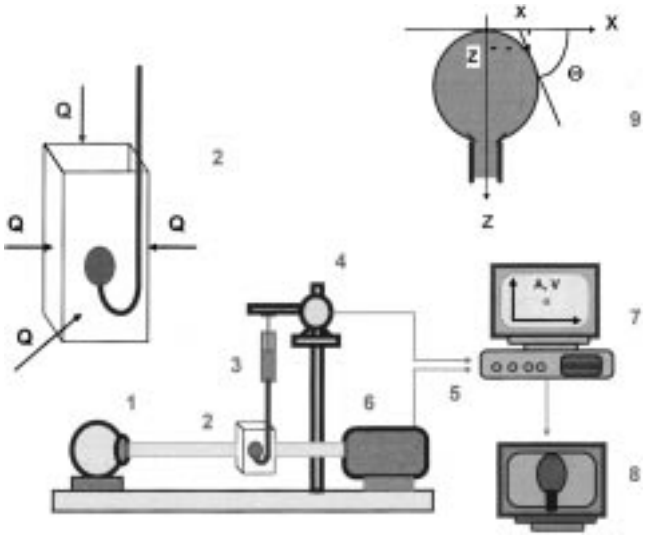

Figure 1. Dynamic drop tensiometer: (1) light source, (2) oil or water drop, (3) syringe, (4) motor, (5) drop profile digitizer, (6) CCD camera, (7) personal computer, (8) monitor, (9) drop profile.

standard accuracy of the interfacial tension is roughly 0.1 mN/m. However, the reproducibility of the results (for at least two measurements) range between 0.5 and 1.5%, with the minimum reproducibility corresponding to higher temperatures.

The surface viscoelastic parameters—such as surface dilational modulus, E, and its elastic, E_d, and viscous, E_v, components—were measured as a function of time, t, amplitude, $\Delta A/A$, and angular frequency, ω. The method involved a periodic automated-controlled, sinusoidal interfacial compression and expansion performed by decreasing and increasing the drop volume, at the desired amplitude ($\Delta A/A$). The surface dilational modulus derived from the sinusoidal change in interfacial tension, σ, (eq 2), resulting from a small sinusoidal change in surface area (eq 3), may be described by eq 4 (Lucassen and van den Temple, 1972).

$$\sigma = \sigma_o \sin(\omega t + \delta) \qquad (2)$$

$$A = A_o \sin(\omega t) \qquad (3)$$

$$E = \frac{\mathrm{d}\sigma}{\mathrm{d}A/A} = -\frac{\mathrm{d}\pi}{\mathrm{d}\ln A} \qquad (4)$$

where σ_o and A_o are the strain and stress amplitudes, respectively, δ is the phase angle between stress and strain, $\pi = \sigma^o - \sigma$ is the interfacial pressure, and σ^o is the interfacial tension in the absence of protein.

The dilational modulus is a complex quantity and is composed of real and imaginary parts (eq 5). The real part of the dilational modulus or storage component is the dilational elasticity, $E_d = |E| \cos \delta$. The imaginary part of the dilational modulus or loss component is the surface dilational viscosity $E_v = |E| \sin \delta$. The ratio (σ_o/A_o) is the absolute modulus, $|E|$, a measure of the total unit material dilational resistance to deformation (elastic + viscous). For a perfectly elastic material the stress and strain are in phase ($\delta = 0$), and the imaginary term is zero. In the case of a perfectly viscous material $\delta = 90°$ and the real part is zero. The loss angle tangent, tan δ, can be defined by eq 6. If the film is purely elastic, the loss angle tangent is zero.

$$E = (\sigma_o/A_o)(\cos \delta + i \sin \delta) = E_d + iE_v \qquad (5)$$

$$\tan \delta = E_v/E_d \qquad (6)$$

The experiments were carried out at temperatures ranging from 20 to 80 °C. The temperature of the system was maintained constant within 0.1 °C at T < 40 °C and within

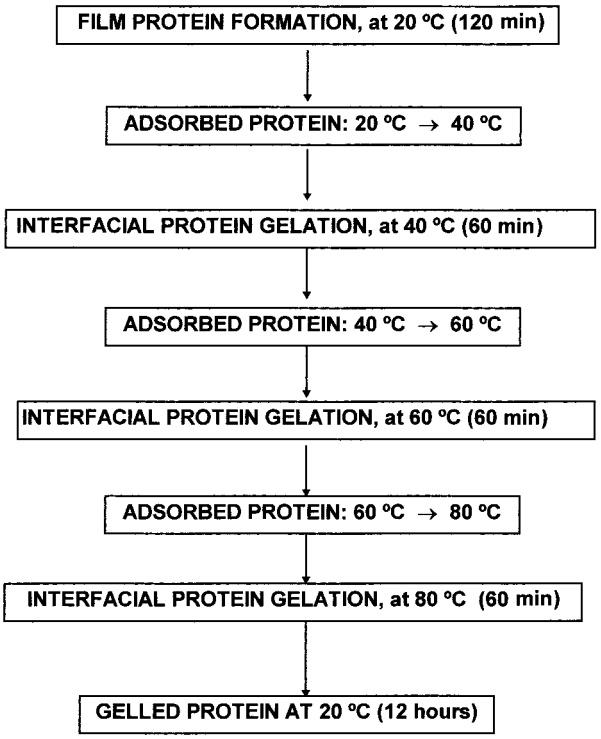

Figure 2. Protocol for consecutive WPI gelation at the oil-water interface.

0.3 °C at $T > 40$ °C by circulating water from a thermostat. The pH and the ionic strength were maintained constant at 5.0 and 0.05 M, respectively, by using a acetic−acetate buffer. Protein solutions at 0.01% w/w were prepared freshly by stirring during 30 min. The protein concentration in the aqueous phase ensures complete monolayer coverage, as deduced from the adsorption isotherm (data not shown). The solution was placed in the syringe or in the cuvette and then in the compartment and was allowed to stand for 30 min to achieve the desired constant temperature. Then either a drop of protein solution was delivered into the oil phase or a drop of oil was delivered into a solution of protein and allowed to stand for 120 min at 20 °C to achieve protein adsorption at the oil−water interface. Afterward, the temperature was increased to 40, 60, or 80 °C, maintaining in each individual experiment the final temperature (40, 60, or 80 °C) constant during 60 min. During the temperature rise and the isothermal treatment the gelation of the protein was monitored by observing the changes both in interfacial tension and in the viscoelastic characteristics of the film.

To analyze the protein gelation at the oil−water interface, the protein solution was placed in the syringe and the oil in the cuvette. Thus, the heat fluxes from the oil to the interface where a previously adsorbed protein film gels as a function of time. After 60 min of gelation at the desired temperature, the temperature of the system was cooled back to 20 °C, and then the interfacial tension and viscoelastic characteristics of the protein film were monitored over time.

In another set of experiments, the protein gelation at the oil−water interface (drop of protein solution) was followed by a continuous increase in temperature according to a protocol (Figure 2). The interfacial tension and the viscoelastic characterization of WPI adsorbed film was monitored during the temperature rise at each isothermal treatment (at 40, 60, and 80 °C) and, finally, at 20 °C after cooling back. Alternatively, WPI solution was placed in the cuvette and was allowed to stand for 120 min at 20 °C to allow protein adsorption at the oil−water interface of an oil drop created at the tip of the syringe immersed in the bulk protein solution. Afterward, the temperature was changed, and the gelation process was monitored as previously described (Figure 2). In this set of experiments the gelation of the protein in solution competes with a previously adsorbed protein film which eventually gels with time.

The microscopic observation (shape and opacity) of the drop after gelation coupled with image analysis could give complementary information about the gel structure, ranging from a particulate network structure—with a weak electrostatic repulsion between protein molecules—to a fine-stranded structure, with strong protein−protein repulsion. The opacity of the drop was measured by means of the gray level of the drop in relation to that of the continuous phase. Clear protein gels are characterized by a high value of the gray level. The shape of the drop after a sudden compression−expansion cycle provides qualitative information about the texture of the protein-gelled film at the oil−water interface.

RESULTS AND DISCUSSION

Effect of Temperature on Interfacial Gelation. The effect of gelation temperature on interfacial pressure and surface rheological properties for WPI films at the oil−water interface adsorbed is shown in Figures 3, 4, and 5. As the heat flux takes place from the oil in the cuvette toward the aqueous protein solution in the drop, through the interface of previously adsorbed protein film, the protein gelation takes place at the oil−water interface. The interfacial tension decreasing during the heat-treatment reflects an increase in surface activity of WPI adsorbed films with time due not only to a typical adsorption process (Rodríguez Patino et al., 1999), but also to structural rearrangement of proteins at the interface caused by the heat-treatment. It can be seen that at the end of the heat-treatment, the interfacial tension of WPI films at 20 °C is lower for a heat-treated protein (at the end of the equilibration time) than for the native protein, with the smallest difference as the heat-treatment takes place at 40 °C (Figure 3), and increasing for heat-treatment at 60 °C (Figure 4) and at 80 °C (Figure 5).

No matter what the temperature of gelation—40 °C (Figure 3), 60 °C (Figure 4), or 80 °C (Figure 5)—the surface dilational modulus decreased during the heating period, passed through a minimum at a temperature close to that of isothermal gelation (40, 60, or 80 °C), then increased during the gelation period at constant temperature and tended to a plateau value just at the end of this period of ~60 min. That is, the maximum value of E was observed at the end of the gelation process at constant temperature: $E = 28.2 \pm 0.3$ (Figure 3), 31.0 ± 0.5 (Figure 4), and 22.2 ± 0.3 mN/m (Figure 5) after 60 min of heat treatment at 40, 60, and 80 °C, respectively. These results indicate that during this period a competition exists between film fluidity with the temperature rising and an increase in the mechanical characteristics probably due to the gelation process. That is, the texture of interfacial gel is not only associated with the heating process used to form the gel but is also dependent on the effect of temperature on the gelled WPI film during surface rheological measurement, as observed for gelation in solution (Ziegler and Foegeding, 1990). We do not exclude changes in rheological properties associated with the temperature.

Afterward, as the drop was cooled back to 20 °C, a significant increase in E was observed. It must be emphasized that the surface dilational modulus, at the first point after cooling back to 20 °C, increased from 28.2 ± 0.3, 31.0 ± 0.5, and 22.2 ± 0.3 mN/m to 69.7 ± 0.8, 126 ± 1.6, and 636 ± 9.5 mN/m, as the temperature

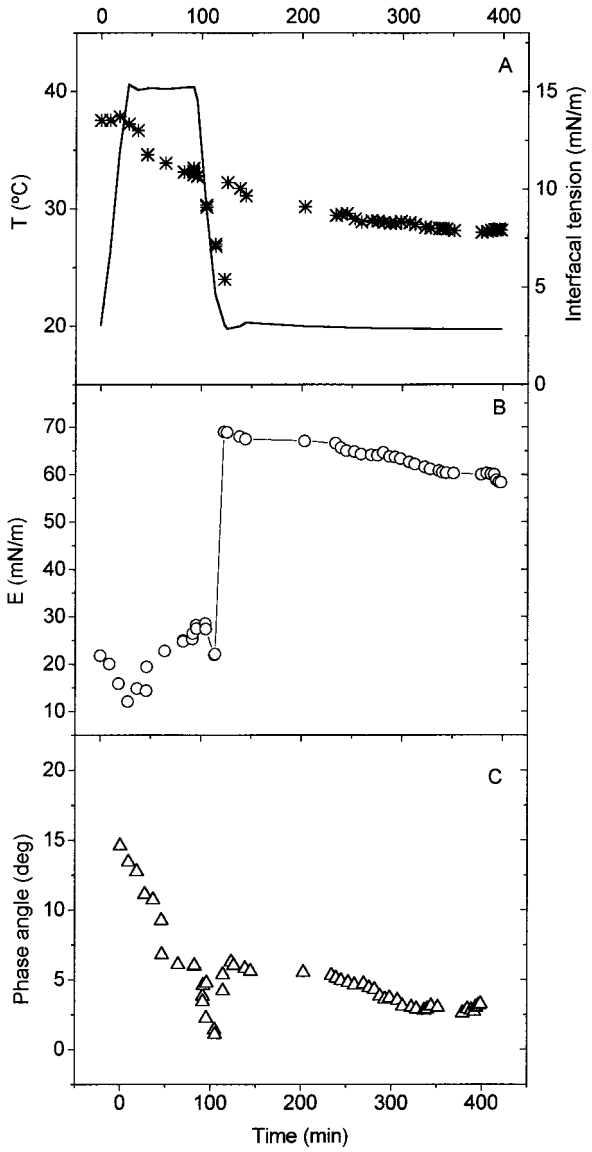

Figure 3. Time evolution of (A) temperature (—) and interfacial tension (*), (B) surface dilational modulus, *E*, and (C) phase angle, for WPI-adsorbed films during heat treatment up to 40 °C. Protein concentration in drop solution: 0.01% w/w, pH 5, I = 0.05 M. Frequency of oscillation: 100 mHz. Amplitude of sinusoidal oscillation: 15%.

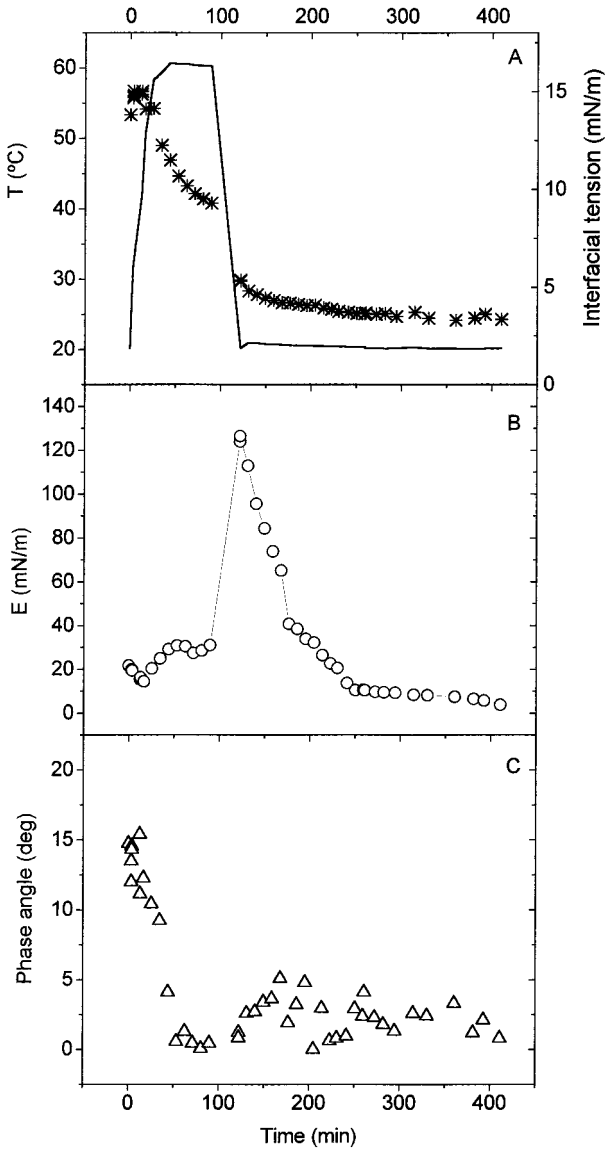

Figure 4. Time evolution of (A) temperature (—) and interfacial tension (*), (B) surface dilational modulus, *E*, and (C) phase angle, for WPI-adsorbed films during heat treatment up to 60 °C. Protein concentration in drop solution: 0.01% w/w, pH 5, I = 0.05 M. Frequency of oscillation: 100 mHz. Amplitude of sinusoidal oscillation: 15%.

decreased from 40 (Figure 3), 60 (Figure 4), and 80 °C (Figure 5) to 20 °C, respectively. This strongly suggests that the gelation became more pronounced upon cooling. The same behavior was observed by Ball and Jones (1995) and Green et al. (1999) for lysozyme- and BSA-adsorbed films on solid substrates by means of AT-FTIR. Finally, a relaxation process took place during the waiting time at 20 °C, and *E* relaxed to a lower value.

The phase angle is an important rheological parameter to characterize the gelation process in the bulk phase (Oakenfull et al., 1997; Ziegler and Foegeding, 1990). The time evolution of the phase angle during the heat treatment of WPI-adsorbed films at the oil−water interface is included in Figures 3−5 for protein interfacial gelation at 40, 60, and 80 °C, respectively. It can be seen that during the heat-treatment—i.e., heating, gelling period at constant temperature, and further cooling back and waiting time at 20 °C—, the phase angle decreased to a plateau value close to 0°. During the heat treatment, the film behaved typically as viscoelastic with nonzero phase angle, and with increasing elastic characteristics as the heat-treatment progressed. In fact, during the heat treatment the elastic component of the modulus increased at the expense of the viscous component. At the end of the gelation process, after a waiting time at 20 °C, the film behaved as purely elastic. These results in addition to *E* evolution with the gelation process strengthens the hypothesis that the interfacial gelation becomes more pronounced upon cooling.

The results in Figures 3−5 represent the first viscoelasticities reported for gelation of a protein adsorbed at the oil−water interface. It must be emphasized that thermal denaturation of WPI at the oil−water interface began to aggregate at a temperature lower than that observed for aggregation in solution (Ball and Jones, 1995; Clark et al., 1981; Phillips et al., 1994; Kinsella et al., 1994), a phenomenon similar to that observed for lysozyme- and BSA-adsorbed films on solid substrates (Ball and Jones, 1995; Green et al., 1999). An explana

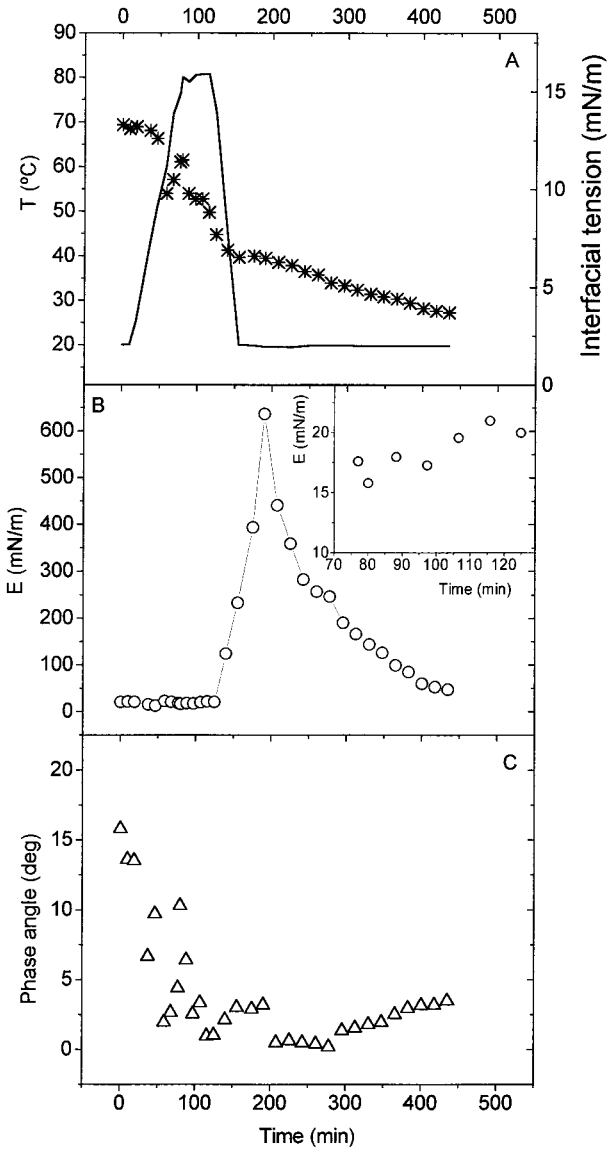

Figure 5. Time evolution of (A) temperature (—) and interfacial tension (*), (B) surface dilational modulus, *E*, and (C) phase angle, for WPI adsorbed films during heat treatment up to 80 °C. Protein concentration in drop solution: 0.01% w/w, pH 5, I = 0.05 M. Frequency of oscillation: 100 mHz. Amplitude of sinusoidal oscillation: 15%.

tion for the differences observed between bulk and adsorbed WPI in relation to the heat treatment is due to the fact that the protein concentration in the adsorbed film is higher than that in solution which may facilitate protein−protein interactions and aggregation at the interface, giving a gelled film. Thus, although the bulk concentration may be too low for gelation in solution (Cayot and Lorient, 1997; Oakenfull et al., 1997; Ziegler and Foegeding, 1990), the interfacial concentration in excess—as detected by the significant reduction in interfacial tension (Figures 3−5)—would be enough to allow WPI gelation even at low temperature (i.e., at 40 °C in our experiments).

Microscopic observation of the drop gives complementary information to support such a hypothesis. Figure 10 shows WPI drops at 20 °C (Figure 10A) and after the heat treatment at 40 (Figure 10B), 60 (Figure 10C), and 80 °C (Figure 10D). It can be seen that, even with the heat-treatment at 40 °C, the drops lose the Laplacian shape (see Figure 10B) with the heat treatment,

Table 2. Opacity of the Drop (I_1) Measured by Means of the Gray Level in Relation to That of the Continuous Phase at Two Representative Points (I_{o_1} and I_{o_2}) for Heat-Treated WPI-Adsorbed Films after Cooling Back to 20 °C (See Figure 10C)[a]

heat treatment T (°C)	I_{o_1}	I_{o_2}	I_1
40[b]	81.0	82.0	81.5
60[b]	78.8	81.5	80.2
80[b]	79.0	80.0	80.2
40[c]	76.0	80.4	77.4
60[c]	76.0	80.4	77.9
80[c]	66.0	65.8	68.0
80[d]	67.0	68.0	28.8

[a] The scale range was between 0 (minimum light intensity for a black drop) and 255 (maximum light intensity for a white drop) [b] Heat treatment of WPI at the oil−water interface. [c] Heat treatment of WPI in the bulk phase. [d] Heat treatment of WPI in the bulk phase at 80 °C, after 12 h of waiting time at 20 °C. The values are the average of ten measurements.

giving an extended pear-like drop shape. In fact, a deformation is produced in the drop surface during heating, giving a rigid film as the drop is cooled at 20 °C, a behavior that we associated with the interfacial gelation of the adsorbed protein films. As a consequence of interfacial gelation, the film presents irregularities which can be measured by the profile of the light at a plane horizontal to the drop. The peaks observed in the light profile are an indication of the existence of folds in the film after the heat treatment (Figure 10). The drop deformation is higher and more time-dependent as the gelation temperature increases at 80 °C. Table 2 presents data on gray level values of the drop irrespective of the temperature of the continuous oil phase. From the data in Table 2 it can be concluded that WPI gives transparent gelled films at the oil−water interface with heat-treatment, no matter what the gelation temperature.

On the other hand, the loss of the Laplacian shape upon heating introduces important experimental problems as the drop profile is processed in order to obtain the interfacial pressure and surface dilational properties. Thus, to obtain absolute, not apparent, data on interfacial characteristics of gelled adsorbed film, the frontier for the drop analysis must be situated according to a laplacian drop shape (see horizontal line in Figure 10). This experimental precaution is more important as the gelation process takes place at 80 °C because the drop volume close to the tip of the syringe did not return to its original shape during a compression/expansion cycle at the working frequency (100 mHz).

Consecutive WPI Gelation at the Oil−Water Interface. As in previous experiments, the protein gelation takes place at the oil−water interface of an aqueous solution of protein in the drop. But, in this experiment the gelation takes place consecutively at 40, 60, and 80 °C, according to the protocol shown in Figure 2. The effect of gelation temperature on interfacial tension and surface rheological properties for consecutive heat-treatment at 40, 60, and 80 °C during 60 min is shown in Figure 6. The heat-treatment of WPI-adsorbed films at the oil−water interface produced significant changes in the interfacial tension (Figure 6A), dilational modulus (Figure 6B), and phase angle (Figure 6C). The interfacial tension decreased during the heat treatment, which reflects an increase in surface activity of WPI adsorbed films with time due the combined effect of protein adsorption (Rodríguez Patino et al., 1999) and heat treatment, as discussed in the

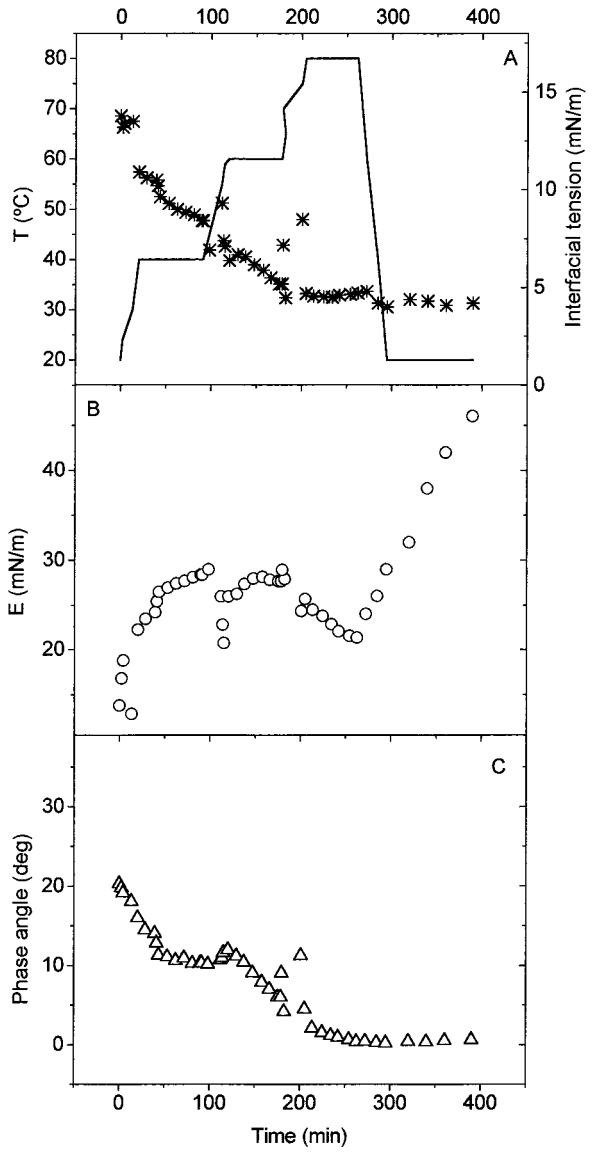

Figure 6. Time evolution of (A) temperature (—) and interfacial tension (*), (B) surface dilational modulus, *E*, and (C) phase angle, for WPI-adsorbed films during consecutive heat treatment at 40, 60, and 80 °C. Protein concentration in drop solution: 0.01% w/w, pH 5, $I = 0.05$ M. Frequency of oscillation: 100 mHz. Amplitude of sinusoidal oscillation: 15%.

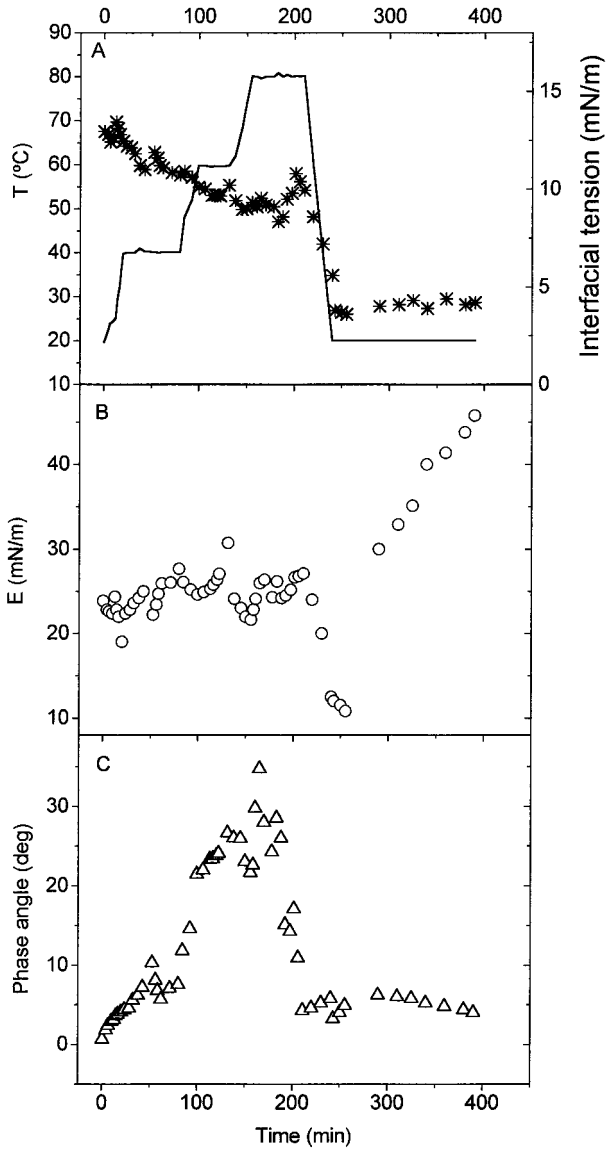

Figure 7. Time evolution of (A) temperature (—) and interfacial tension (*), (B) surface dilational modulus, *E*, and (C) phase angle, for WPI-adsorbed films during consecutive heat treatment at 40, 60, and 80 °C. Protein concentration in solution: 0.01% w/w, pH 5, $I = 0.05$ M. Frequency of oscillation: 100 mHz. Amplitude of sinusoidal oscillation: 15%.

preceding section. It must be emphasized that at the end of the heat treatment the interfacial tension of WPI films at 20 °C was only ~4 mN/m.

As observed in previous experiments, *E* decreased during the heating period, passed through a minimum at a temperature close to that of isothermal gelation (40 and 60 °C), and then increased during the gelation period at constant temperature and tended to a plateau value just at the end of this period of ~60 min. (Figure 6B). The behavior during the heat-treatment at 80 °C is different. During the heat-treatment at 80 °C, *E* decreases monotonically toward a minimum. These results corroborate the idea that during this period a competition exists between the film fluidity at increasing temperature and an increase in the film mechanical characteristics probably due to the gelation process. During the cooling back to 20 °C, and especially during the waiting time at 20 °C, *E* increased ultimately to a value which was practically 3 times higher than that for a nonheat-treated WPI-adsorbed film at the end of the equilibration time. During the overall period the film behaved as viscoelastic with an elastic component which was practically similar to the modulus and a viscous component which decreased with the heat treatment. At the end of this period the viscous component was practically zero. That is, the film behaved as purely elastic over the frequencies range studied.

Consecutive WPI Gelation in the Bulk Phase. In contrast to experiments described in previous sections, in this set of experiments the protein gelation takes place in the bulk phase and then at the oil−water interface of a oil drop suspended in an aqueous solution of protein. The gelation takes place consecutively at 40, 60, and 80 °C, according to the protocol shown in Figure 2. The results in Figure 7 show qualitatively but not quantitatively the same features as for protein gelation at interface (Figure 6). The heat treatment of WPI in solution produces significant changes in the interfacial tension (Figure 7A), surface dilational modulus (Figure 7B), and phase angle (Figure 7C) of the adsorbed WPI

film. The interfacial tension decreased, and the surface dilational modulus shows the same time dependence during the heat treatment as discussed in preceding sections. However, the viscoelastic characteristics of WPI gelation in the bulk phase are lower as the WPI gelation takes place in the bulk phase than for the interfacial gelation.

The microscopic observation of the gelled films also showed important differences depending on whether the WPI gelation takes place at the oil−water interface or previously in the aqueous bulk phase. The drop surface does not present any fold after continuous isothermal heat treatment at 40, 60, and 80 °C. Figure 10E shows a drop after the heat-treatment at 80 °C, as an example. The heat-treated WPI film forms a network at the interface due to de adsorption of gelled protein from the bulk aqueous phase. The adsorption of gelled WPI at the interface produces nonclear cloudiness in the drop surface. However, the most important difference in the adsorption of previously gelled WPI, in relation to the interfacial gelation of WPI discussed in the previous section, is due to the fact that the film of previously gelled WPI in solution decreased significantly the light intensity in relation to the value of the continuous phase. It can be seen (Table 2) that the relative intensity is reduced significantly, especially for WPI-gelled adsorbed film at the higher temperatures after 12 h of waiting time at 20 °C.

These results indicate that the conformation of proteins at interface after the heat treatment should be different, depending on the conditions in which the gelation process takes place, either that produced directly at the oil−water interface or that produced in the bulk aqueous phase with a further repercussion on the viscoelastic characteristics of the protein film. Clearly, the gelation of WPI at the oil−water interface depends on the conditions at which the gelation process takes place.

Viscoelastic Characteristics of WPI-Gelled Films. Figures 8 and 9 show the effect of the amplitude (at three representative frequencies of 20, 50, and 100 mHz) and frequency of the sinusoidal area fluctuations (at an amplitude of 15%) on surface rheological properties (i.e., surface dilational modulus and phase angle), respectively, for WPI-adsorbed films after heat treatment at 80 °C for 60 min and then cooling at 20 °C, as an example. The surface dilational modulus (Figure 8A) increased with the amplitude of deformation to a maximum at an area amplitude of ∼10%, and then decreased as the amplitude progressed. That is, over the range of area amplitudes we studied the gelled WPI films at the oil−water interface did not present any linear viscoelastic regime, with a significant effect of amplitude on the surface dilational modulus. It must emphasized that E decreased from 43 to 54 mN/m to 13−17 mN/m as the amplitude increased from 10 to 30%. This dependence may be attributed to reorganization changes in the interfacial WPI gel with the compression/expansion amplitude, with a significant effect at the amplitude range of 10−30%, probably due to destruction of the gelled film structure by the large deformation.

However, the phase angle (Figure 8B) varied from 0 at a amplitude of ∼4% to a maximum of 4−4.5° at 10% of amplitude and then decreased to a plateau value of 2−2.5° at the highest amplitudes. At lower area deformations WPI gelled films behaved as purely elastic with

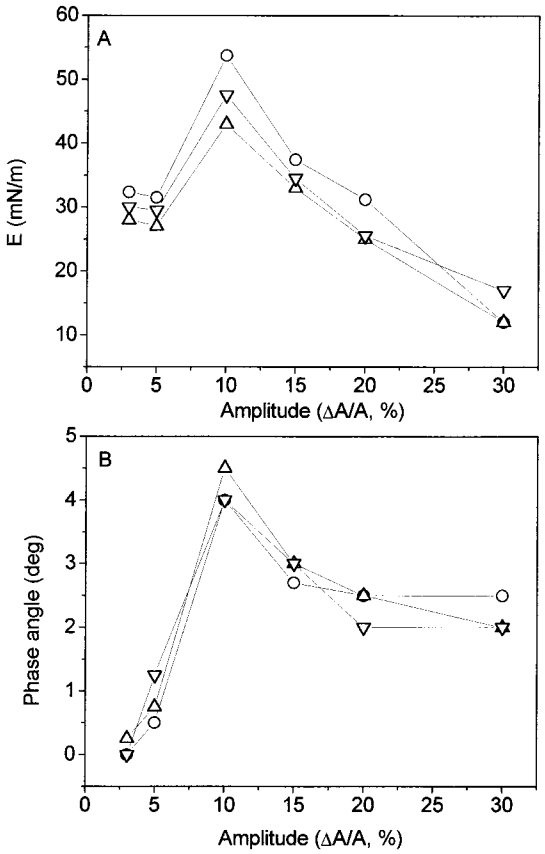

Figure 8. Amplitude dependence of (A) surface dilational modulus and (B) phase angle, at three representatives oscillation frequencies (○: 20 mHz, △: 50 mHz, and ▽: 100 mHz), for a adsorbed WPI film after consecutive heat treatment at 40, 60, and 80 °C for 60 min and then cooled at 20 °C, and after 12 h of equilibration time. Temperature: 20 °C. Protein concentration in solution: 0.01% w/w, pH 5, I = 0.05 M.

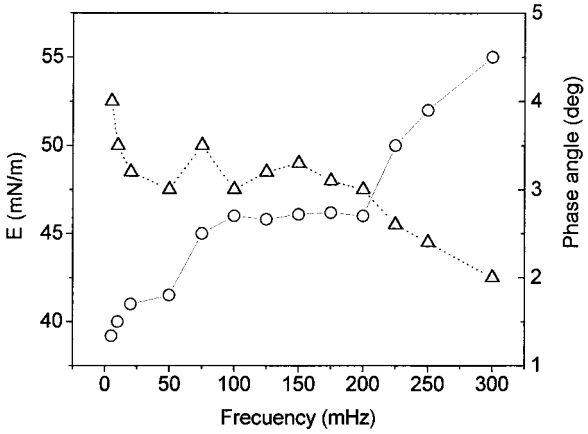

Figure 9. Frequency dependence of (○) surface dilational modulus, E, and (△) phase angle for a previously gelled WPI film after consecutive heat treatment at 40, 60, and 80 °C for 60 min and then cooled at 20 °C, and after 12 h of equilibration time. Amplitude of sinusoidal deformation: 15%. Temperature: 20 °C. Protein concentration in solution: 0.01% w/w, pH 5, I = 0.05 M.

the onset of the viscoelastic behavior at 4% of area deformation as shown by the nonzero viscous phase angle. However, even over this range of higher amplitudes, the viscoelastic characteristics of WPI-gelled films were almost purely elastic because the low values of phase angle gave low values of the viscous component and an elastic component which was practically similar to the surface dilational modulus (data not shown).

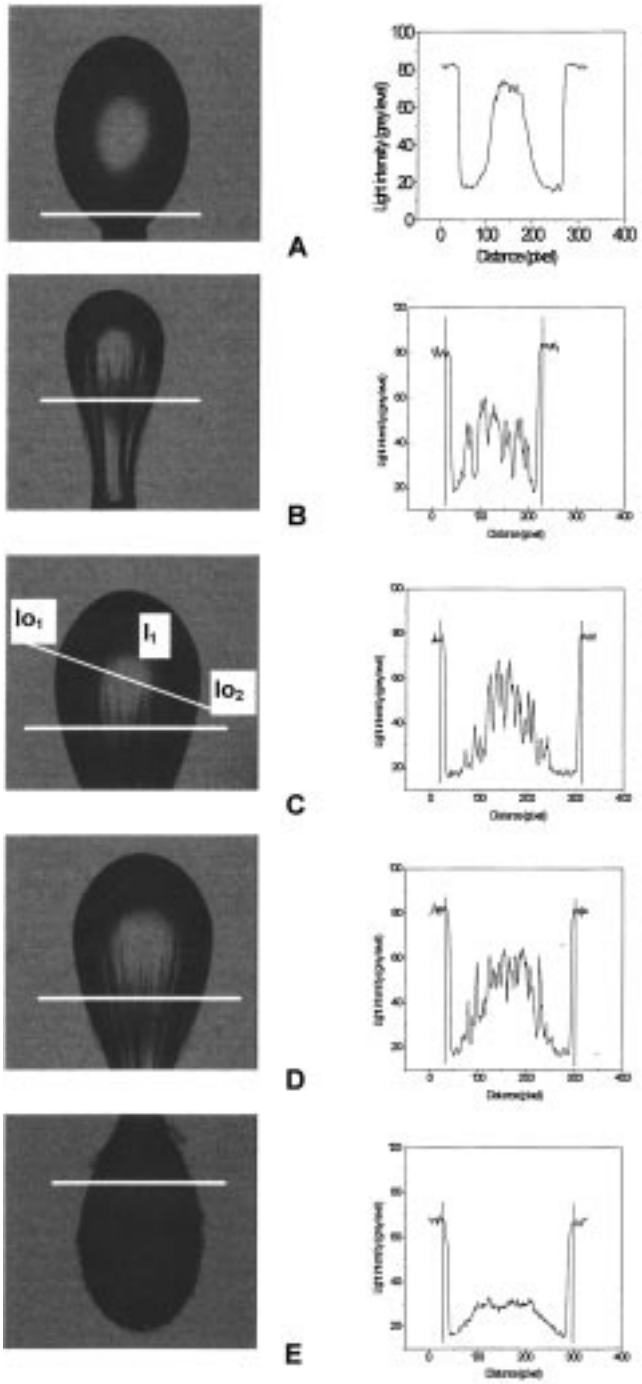

Figure 10. Microscopic observation (shape and opacity) of drops of heat-treated WPI film at the oil−water interface, at (A) 20, (B) 40, (C) 60, and (D) 80 °C. (E) Microscopic observation (shape and opacity) of a drop of heat-treated WPI in the bulk phase at 80 °C. Protein concentration in solution: 0.01% w/w, pH 5, $I = 0.05$ M. The profile of the light intensity (light intensity versus distance) across the horizontal plane (indicated in the drop images A−E by a horizontal line) is included in the right-hand side of each drop image.

Figure 9 shows complementary data on the viscoelastic behavior of WPI-gelled films. The frequency dependence of surface dilational modulus and phase angle, the so-called mechanical spectrum in bulk rheology, is characteristic of viscoelastic films (Lucassen-Reynders and Benjamins, 1999) with lower E and higher phase angle at lower frequencies. However, the opposite trend was observed with higher modules and lower phase angle at the highest frequencies. That is, the high modulus values in the range of high frequencies is mainly due to the elastic characteristics of the film because the phase angle was minimum and close to zero in this high-frequency regime. The relaxation phenomena associated with this frequency dependence may be rather complex, including reconformation of WPI molecules gelled at interface taking place at the higher frequencies (i.e. 100−300 mHz) with a time scale of ∼3−10 s. In addition, as the modulus was affect by a decrease in frequency down to 5−20 mHz, partial film desorption/adsorption during the compression/expansion cycle and, especially, relaxation phenomena due to film collapse, could be responsible for the viscoelastic behavior of WPI-gelled films with a time scale of 50−200 s—a time scale characteristic for relaxation phenomena in a saturated protein monolayer at the air−water interface at the highest interfacial pressures (Rodríguez Niño et al., 1999).

CONCLUSIONS

In this work we have analyzed for the first time the interfacial tension and viscoelastic characteristics of WPI heat-induced gels at the oil−water interface as a function of temperature and heating conditions. Heat-induced interfacial aggregation of a whey protein isolate (WPI) with a high content of β-lactoglobulin, previously adsorbed at the oil−water interface, was studied by interfacial dynamic characteristics performed in an automatic drop tensiometer, a noninvasive technique, coupled with microscopic observation (shape and opacity) and image analysis of the drop before and after heat treatment. The experiments were carried out at temperatures ranging from 20 to 80 °C with different thermal regimes (interfacial isothermal gelation at 40, 60, and 80 °C, consecutive interfacial gelation at 40, 60, and 80 °C, and consecutive gelation of protein in solution at 40, 60, and 80 °C). In each case the effect of cooling back to 20 °C on previously heat-treated WPI films was also analyzed. During the heating period a competition exists between the effect of temperature on the film fluidity and the increase in mechanical properties (especially the surface dilational modulus and its elastic component) associated with the interfacial gelation process. During the isothermal treatment the surface dilational modulus increases, and the phase angle decreases with time to a plateau value. The frequency dependence of surface dilational modulus and phase angle are characteristics of viscoelastic films with increasing phase angle and decreasing surface dilational modulus at the lower frequencies. Over the range of area amplitudes studied (3−30%) the heat-treated WPI films do not present any linear viscoelastic regime. However, the effects of heat-treatment on surface dynamic properties of WPI-adsorbed films depends on the conditions at which the gelation process takes place, either that produced directly at the oil−water interface or that produced in the bulk aqueous phase with a further repercussion on the interfacial characteristics of the WPI films. The microscopic observation of gelled films gives complementary information on the effect of the heat-treatment on WPI adsorbed films, depending on whether the WPI gelation takes place at the oil−water interface or previously in the aqueous bulk phase. As the surface concentration of an adsorbed WPI film is higher than that in bulk solution, the interfacial gelation is possible at a protein concentration in solution (0.01% w/w) far lower than that necessary for

gelation in solution. Finally, what this study clearly established is that dynamic drop tensiometry coupled with drop image analysis is a useful technique for analyzing the interfacial gelation of WPI-adsorbed films at the oil−water interface. The effect of protein concentration in solution on interfacial gelation will be studied in a forthcoming paper.

From a practical point of view, these data clearly demonstrated that there exists a close correlation between interfacial gelation of WPI-adsorbed film at the oil−water interface and protein surface coverage, surface shear viscosity, and stability (against creaming, coalescence, and flocculation) of oil-in-water emulsions stabilized by WPI (Demetriades et al., 1997; Dickinson and Hong, 1994, Dickinson and McClements, 1995; Monahan et al., 1966). In addition, the stability and physicochemical properties of WPI-stabilized emulsions were particularly sensitive to the thermal history (Demetriades et al., 1997), as observed in the heat treatment of WPI-adsorbed film in this study.

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