Effect of pH and Protein Concentration on Rheological Behavior of Acidic Soybean Protein Gels

María Cecilia Puppo† and María Cristina Añón*

Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), 47 y 116 (1900) La Plata, Argentina

Heat-induced gels of different pH and protein concentration were obtained from pH 3.25 and 8.0 soybean isolates. Viscoelasticity and texture behavior of the gels were analyzed by dynamic rheological assays and uniaxial compression tests. Results indicate that soybean protein dispersions exhibit a viscoelastic behavior. At low protein concentration, the acidic dispersions behave as pseudogels. Elasticity and resistance to deformation increase, and fracturability and hardness decrease as pH decreases. Acidic gels of high protein concentration behave as strong or true gels. The latter show elasticity, fracturability, hardness, and resistance to deformation prior to rupture values higher than low protein concentration gels.

Keywords: *Acidic soybean protein gelation; heat-induced gelation; gel texture properties; gel viscoelasticity*

INTRODUCTION

Proper understanding of food behavior requires thorough knowledge of its structure, the arrangement and interactions of its structural elements, and those interaction forces that determine the consistency and physical stability of the products (Heertje, 1993).

Protein gels are composed by a protein chain matrix within which the aqueous phase is occluded (Stading et al., 1993). Some gels present a nonhomogeneous structure with high- and low-density zones, possibly owing to differences in chain type and intercrossings and/or to the formation of pores of different size. Such inhomogeneities would originate during the gelation process or after it, especially if the material formed is not a stable gel (Stading et al., 1992, 1993). If gelation takes place before precipitation, a homogeneous elastic matrix will be formed, otherwise repulsion forces may predominate leading to the microsyneresis phenomenon giving rise to matrixes having polymer-rich zones and regions with lower polymeric density (Stading et al., 1992).

Rheological properties such as viscoelasticity and texture are closely related with protein gel microstructure (Beveridge et al., 1984). Hardness of soybean protein gels depends on the type of matrix formed (Utsumi and Kinsella, 1985a,b). The gels of fine-stranded matrix (Foegeding et al., 1995) are harder and retain more water than those of more open matrixes (particulate gels). The large pores of the particulate gels inhibit its capacity to inmobilize water via capillary forces (Bowland and Foegeding, 1995). Besides, gel hardness increases with the sulfhydryl−disulfide interchange, which is favored at increasing protein concentration. In this regard, Zheng et al. (1993) have indicated that the contributions of covalent and noncovalent bonds to gel texture and viscoelasticity are different. Disulfide bonds play an important role in stabilizing the gel and in increasing gel matrix hardness whereas hydrogen and hydrophobic interactions are especially responsible for keeping the structure and for viscosity increase. Different thermal treatments and interaction of soybean proteins with other components give rise to a vast variety of gels responsible of the textural quality of foods (Kang et al., 1991). Several studies of viscoelastic (Kohyama and Nishinari, 1992, 1993; Nagano et al., 1994a,b; Ker et al., 1992; Yoshida et al., 1992; Kohyama et al., 1992; Nishinari et al., 1991) and textural (Utsumi and Kinsella, 1985a,b; Wang and Damodaran, 1990, 1991) properties of thermally treated soybean proteins (isolates, 7S and 11S fractions) of neutral pH (7.5−8.0) have been performed.

Little knowledge has been accumulated yet on the gelation properties of soy proteins for pH below the pI (4.5). By studying them, it would be possible to find different gels from conditions, which are important for formulating acidic semisolid foods. Gel hydratation properties and matrix structure at different acidic pH and protein concentration had been studied in a previous work (Puppo et al., 1995) whereas the aim of the present work was to deepen the study of the influence of pH and protein concentration on viscoelastic and textural properties of acidic soy protein gels.

MATERIALS AND METHODS

Preparation of Soy Protein Isolates. Soy protein isolates were prepared from defatted flour produced by Santista Alimentos S. A. (Brazil). Proteins were obtained by alkaline extraction (pH 8.0) of the flour and subsequent precipitation at the isoelectric point (pI = 4.5), as described by Puppo et al. (1995). The isoelectric precipitate was dispersed in distilled water and divided into two subsamples, one for the acidic isolate where pH was brought to 3.25 with HCl 2 N and the

* Corresponding author. Member of the Research Career of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

† Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

S0021-8561(97)01092-3 CCC: $15.00
Published on Web 07/11/1998

other for the basic isolate, using NaOH 2 N up to pH 8.0. The resulting dispersions were freeze-dried.

Various acidic dispersions were prepared from the pH 3.25 isolate by adjusting pH with either HCL 1 N or NaOH 1 N as needed.

Dynamic Tests. Viscoelasticity Determinations. Tests were carried out in a Haake CV20 rheometer using a 1 mm gap parallel-plate sensor. The dispersion was placed on the lower plate, which was thermostated for gelation. Low-viscosity silicone was added around the plate edges to prevent dehydration and to permit a total contact between sample and silicone. The equipment was driven through the Haake software osc. 2.0. The experimental procedures allowed recording of the development and demise of complex modulus (G^*), storage modulus (G'), loss modulus (G''), tan δ (G''/G'), and complex viscosity (η^*) as functions of time and frecuency of oscillation.

The dispersions prepared from pH 3.25 and 8.0 soy protein isolates were tested in the rheometer by deformation scans conducted at 60, 70, 80, and 90 °C. In all tests, a deformation of 10% was determined within the linear viscoelasticity range. Sample frequency scans (10% deformation), after heating the samples for 30 min, were done. The variation of G' and G'' ($f = 1$ Hz) as a function of the thermal treatment temperature was also determined.

Dispersions in distilled water (10% w/w) at pH 2.75, 3.50, and 8.0 were prepared and thermally treated at 90 °C for 30 min. The linear viscoelasticity range was determined in all of them, measuring G^* as a function of deformation. From these results, frequency and time scans of all samples were conducted at the same deformation (d = 10%), within the linear range.

The other set of 10% w/w dispersions were prepared in distilled water at pH 2.50, 2.75, 3.00, 3.25, 3.50, and 8.0 and 14% (w/w) dispersions at pH 2.75, 3.50, and 8.0. After being thermally treated at 90 °C for 30 min, frequency scans were carried out at the same temperature. The rheological behavior was studied by comparing the G' and G'' frecuency dependence with empirical models.

The heating rate was determined by measuring the variation of G' (1 Hz, 10% deformation) as a function of treatment time (90 °C) in pH 2.75, 3.50, and 8.0 dispersions prepared at 10% w/w in distilled water. Experimental data were fitted by the following empirical formula (Nishinari et al., 1991; Yoshida et al., 1992):

$$G'(t) = G'_{sat}\,[1 - \exp(-kt)] \qquad (1)$$

where G'_{sat} is the saturation storage modulus, k the thermal treatment rate constant, and t the time. Constant k was obtained by the nonlinear model method using the SYSTAT (1990) software (SYSTAT, Inc., Evanston, IL).

Compression Tests. *Preparation of Gels.* Dispersions in distilled water of 10 and 14% w/w protein concentration from the pH 3.25 and 8.0 isolates were prepared. The pH of acidic dispersions was then adjusted to 2.50, 2.75, 3.00, 3.25, and 3.50 with HCl 1 N or NaOH 1 N as needed.

All samples were partially deaerated by centrifugation at 1000*g* for 1 min at 15 °C and carefully resuspended using a glass rod. The dispersions were placed in glass tubes (length, 6 cm i.d., 2.2 cm) closed in both ends with hermetic-seal rubber plugs. Gelation was carried out by heating the tubes at 90 °C for 30 min and then immediately cooled in a water bath at 15 °C. The gels were then kept at 4 °C for 24−48 h to ensure complete gelation (Puppo et al., 1995).

Determination of Texture. Gels were compressed to 80% of their original height (3 cm) until rupture (Lee and Batt, 1993), by means of an Instron model 1101 press. To this end, a 50 N cell was used at ambient temperature, the head deformation rate being 100 mm/min (Stamponi and Noble, 1991, 1992; Hsieh et al., 1993; Bertola et al., 1996). In each determination, a minimum of four gels was used, and average values were calculated.

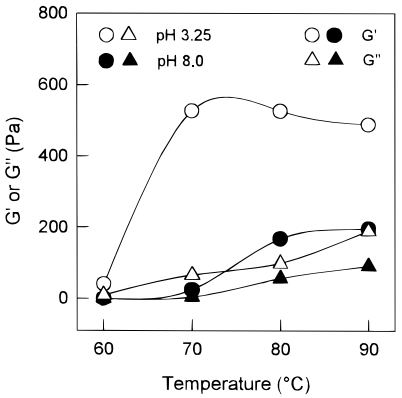

Figure 1. Variation of G' (●, ○) and G'' (▲, △) with temperature for (10% w/w) dispersions at pH 3.25 (○, △) and pH 8.0 (●, ▲).

A one-cycle uniaxial compression test was carried out using parallel plates without lubrication. The rupture strength or fracturability (F) and hardness (H) were determined from the force−time curves. Fracturability was defined as the force of the significant break in the force−time curve, while hardness is the hight of the force peak (Andersson et al., 1973; Bourne, 1982). The engeneering strain or strain at fracture (ϵ_f) was determined from the curves as $\epsilon_f = \Delta L_f/L_0$ where L_0 is the initial height of the sample, $\Delta L_f = L_0 - L_f$, and L_f is the height of the sample at fracture.

Assuming incompressible samples, the force deformation curves were transformed into true stress−true strain curves. The true strain (ϵ_t) was expressed as the logarithmic deformation (Peleg, 1977; Nussinovitch et al., 1989, 1990):

$$\epsilon_t = \ln\frac{L_0}{L_0 - \Delta L} \qquad (2)$$

The true stress (σ_t) was calculated:

$$\sigma_t = \frac{F}{a_0}(1 - \Delta L/L_0) \qquad (3)$$

where F is the force at time t and a_0 is the initial cross-section area.

The initial slope of the true stress−true strain curves, defined as the deformability modulus (E_D) (Nussinovitch et al., 1990), was determined.

The data were statistically analyzed by Analysis of Variance (ANOVA) using the SYSTAT software (1990) (SYSTAT, Inc., Evanston, IL). The significance of differences among results of the several treatments were studied by the Tukey test at $p < 0.05$.

RESULTS AND DISCUSSION

Viscoelasticity of Dispersions. *Effect of Gelling Temperature.* Figure 1 shows the variation of G' and G'' with temperature (f = 1 Hz). Both in the acidic dispersion and in that at pH 8.0, the elastic (G') and viscous (G'') moduli are, at 60 °C, very low, close to zero. As the temperature increases, G' becomes higher than G'', the difference being more important in the acidic sample. The results indicate that pH 3.25 dispersions become more elastic than viscous for temperatures above 60 °C, whereas at alkaline pH, the elastic modulus increases from 70 °C. At higher temperatures, G' values in acidic dispersions are five times as high as those of corresponding alkaline dispersions. Values of G'' are similar for both samples in the entire temperature range, thus evidencing no substantial changes in the viscous component with temperature. The pronounced change in G' between 60 and 70 °C underwent

by the pH 3.25 dispersion is in agreement with denaturation temperatures of the soy protein 7S (71.29 ± 1.37 °C) and 11S (66.54 ± 0.47 °C and 80.93 ± 0.06 °C) fractions at that pH value (Puppo and Añón, 1998). At acidic pH and above 70 °C, both globulins are completely denatured so that no variation of elastic modulus with temperature is observed.

The pronounced change in G' between 60 and 70 °C underwent by the pH 3.25 dispersion is in agreement with denaturation temperatures of the soy 7S and 11S globulins fractions at that pH value. In a previous work (Puppo and Añón, 1998), we studied by DSC the thermal stability and the denaturation degree of soybean protein isolates and soybean fractions (7S and 11S) of different pHs. The 7S fraction of pH 3.25 presented a singular endotherm at a denaturation temperature of 71.29 ± 1.37 °C. The 11S globulin (pH 3.25) presented two peaks, at 66.54 ± 0.47 °C and 80.93 ± 0.06 °C, corresponding to the hexameric and dodecameric form of the glycinin, respectively (Puppo and Añón, 1998). At acidic pH and above 70 °C, both globulins are completetly denatured so that no variation of elastic modulus with temperature is observed.

In pH 8.0 gels, G' increases between 70 and 80 °C and keeps rather constant for higher temperatures. The increase takes place in the same range as that involving denaturation of the 7S fraction (78.63 ± 0.30 °C). The 11S fraction denatures at 66.54 ± 0.47 and 80.93 ± 0.06 °C and at 90.06 ± 0.38 °C for pH 3.25 and 8.0, respectively, indicating that gels elasticity would be influenced mainly by β-conglycinin denaturation (Puppo and Añón, 1998). For 15% w/w dispersions of native soy protein isolates, Owen et al. (1992) and Van Kleef (1986) have also observed an increase of G' at 70 °C followed by a transition at 97 °C due to gelling of 7S and 11 S globulins, respectively.

Therefore, according to the experimental results, the temperature chosen here for preparing the gels (90 °C) ensures the complete protein denaturation required for gelation, allowing more elastic gels to be formed.

Effect of pH. The complete gelation process consists of two stages, with a first heating stage to promote denaturation and protein interaction and a second of cooling. In this work, experiments were carried out at the gelation temperature (90 °C) so that we are measuring variations in the dynamic parameters of the process first stage. In this stage, a fraction of proteins experiences denaturation since, at acidic pH, the remaining fraction was already denatured by pH effect before the thermal treatment (Puppo and Añón, 1998).

Figure 2 shows graphs for dispersions prepared at pH 2.75, 3.50, and 8.0. In each, G', G'', and η^* were represented as a function of the oscillation frequency (dynamic spectrum), and it is observed that their behavior obeys the Maxwell model (Haake, 1991). According to Giboreau et al. (1994), the three samples would behave as semidiluted macromolecular dispersions where elasticity increases with the oscillation frequency. Clark and Ross-Murphy (1987) and Ross-Murphy (1987, 1995b) classified these types of samples as structured network systems (pseudogels) represented by dynamic spectra where G' and G'' increase with frequency. In the first zone, G'' is greater than G' but then, after a crossover, G' becomes higher than G'' in the plateau region. The value of η^* decreases linearly for frequencies higher than 0.22 Pa s (Ross-Murphy, 1995b) [in agreement with Ross-Murphy (1995b)]. These

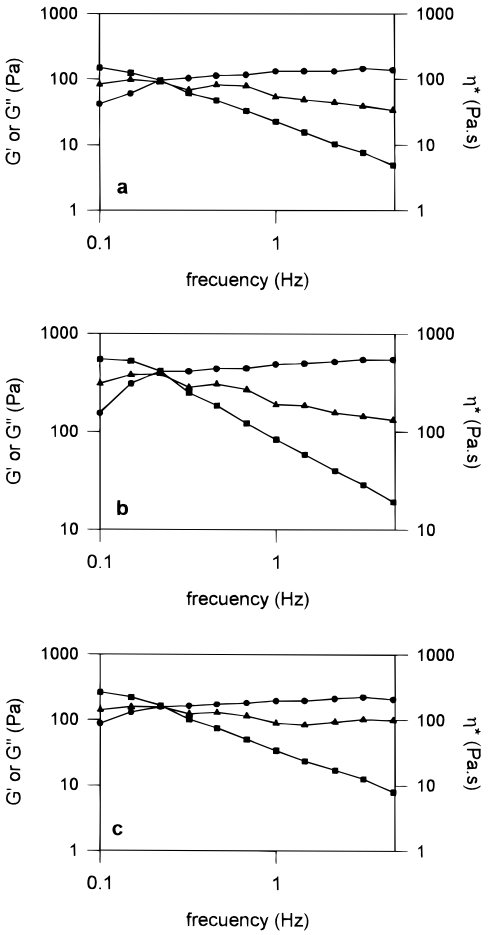

Figure 2. Variation of G' (●), G'' (▲) and η^* (■) with frequency for (10% w/w) dispersions at pH 2.75 (a), 3.50 (b), and pH 8.0 (c).

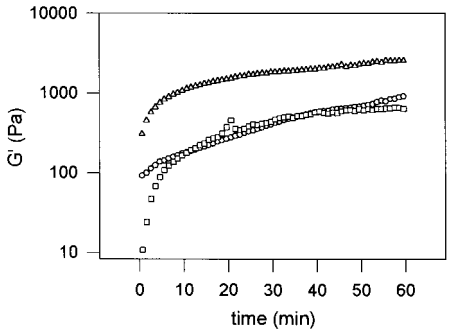

Figure 3. Variation of G' with heating time (90 °C, 30 min) for (10% w/w) dispersions at pH 2.75 (○), 3.50 (△), and 8.0 (□).

systems can be described as "highly elastic solutions", where the phenomenon of association of segments of ordered protein chains is dominant (Cuvelier and Lunay, 1986). This association of protein molecules would be due to noncovalent interactions, mainly of the hydrophobic type (Baird, 1981). If, in these viscoelastic liquids, the parameters were measured after cooling where hydrogen bond interactions are favored, they would possibly show a gellike behavior from a dynamic viewpoint.

The variation of the G^* modulus is similar to that of G' so that the viscoelastic kinetics can be represented in terms of the elastic modulus. For the three 10% w/w dispersions, Figure 3, show that G' increases gradually and continually with time, following a first-order kinetics. A similar behavior was observed for 7S and 11S

soymilk globulins after thermal treatments at 80 °C (Nishinari et al., 1991; Yoshida et al., 1992; Nagano et al., 1994b). This G'−temperature profiles, distinctive of native globular proteins cooperative gelation, have also been described by Van Kleef et al. (1978). The G' values of dispersions prepared at extreme pH are lower than those whose pH is close to the pI. Most of the modulus increase is observed within the first 30 min of thermal treatment, a behavior that justifies gel preparation by heating at 90 °C for 30 min.

By using the model represented by eq 1 for the curves of the pH 2.75, 3.50, and 8.0 dispersions, the values obtained for the gelation rate constant ($k \times 10^4$) were 4.08, 7.55, and 7.17 s^{-1}, respectively ($r < 0.989$). Thus, in the sample of pH closer to the pI, the first gelation stage is faster than it is for samples of extreme pH values. It could then be proposed that during the thermal treatment around the pI value, protein−protein interaction would favor a faster gelation process. Besides, in the pH 8.0 gel, the value of the saturation or equilibrium elastic modulus (G'_{sat}) is reached within the 60 min of heating, whereas, for the pH 2.75 gel, longer treatment time would be allowed for.

The fact that the G'_{sat} value is not reached does not impede gel formation after cooling. Bohlin et al. (1984) have studied the dependency of viscoelastic properties of coagulated milk with time and observed that after cooling the elasticity increased for the gels formed. The transition from sol state, where the molecular mass is finite to the gel state of "infinite" molecular mass occurs at a particular cross-linking degree termed "gel point". Gel equilibrium modulus (G'_{sat} in our case), sometimes associated to gel strength, is proportional to the number of elastically active chains of the matrix (Ne) which, in turn, depends on matrix functionality (f) and cross-linking degree (α). The dependency of G with time presents, in some cases, a lag period, and the gel or gelation time can be estimated from the G vs time curve when G becomes measurable (Ross-Murphy, 1995a).

Thermal treatment at high temperature would favor two opposite phenomena: (a) rupture of hydrogen bonds and decrease of electrostatic interactions, thus weakening intermolecular interactions, and (b) stabilization of hydrophobic interactions which favor molecular association in a three-dimensional structure. The value of G' results from this balance; if the first phenomenon predominates, the elastic modulus will be small, whereas if the second is dominant, G' reaches higher values (Chronakis, 1996). On the basis of the above-mentioned fact, it may be deduced that thermal treatment of soy protein dispersions, either acidic or alkaline (Figure 3), would enhace hydrophobic bonds between protein molecules.

To analyze how dynamic parameters vary with the gel variables, we have chosen a frecuency value (1 Hz) for which measurements are fast enough, while giving a slight fluctuating shear stress response to the deformation applied.

In the acidic pH range, storage (G') and loss (G'') moduli increase, the raise being more pronounced for G' (Figure 4a). In the more acidic gels (pH 2.50), G' and G'' values are similar, and there is an increasing difference of both moduli as pH increases. The pH 8.0 gel exhibits an elastic moduli value intermediate compared to those presented by pH 3.00 and 3.25 gels (Figure 4a).

Viscoelasticity is represented, according to Cooney et al. (1993), by the tangent of the deformation angle (tan $\delta = G''/G'$) (Figure 4b). All tan δ values are below 0.5, revealing that gel behavior is primarily elastic, especially in gels at pH below 3.25. Changes in tan δ are small at increasing gel pH.

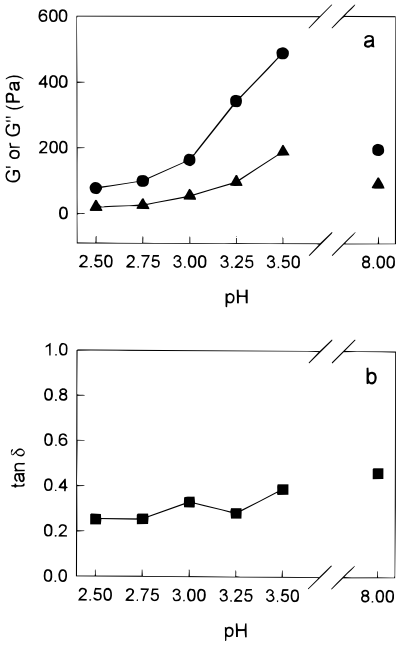

Figure 4. Variation of G' (●) and G'' (▲) (a), and tan δ (■) (b) as a function of gel pH (10%w/w).

Effect of Protein Concentration. While a 10% w/w gel comports, under the conditions tested, as a Maxwellian viscoelastic liquid, the increase of protein concentration up to 14% w/w leads to a viscoelastic solid behavior that follows the Kelvin Voight model (Haake, 1991). The elastic modulus of the latter gel is higher than that of the former, and its value keeps constant over the frequency range tested (Figure 5). The most protein concentration gels exhibit a "gel-like" behavior that reflects the existence of a three-dimensional matrix which is stable at high oscillation frequencies (Clark and Ross-Murphy, 1987; Giboreau et al., 1993). For alkaline gels, this stability would be related to the formation of disulfide bonds by approximation of protein molecules (Giboreau et al., 1993; Chronakis et al., 1995). A linear decrease of η^* is observed as the frequency increases (Figure 5), a typical feature of strong gels (Ross-Murphy, 1995b). Similar results were obtained in soy protein dispersions at different concentrations by Baird (1981) (20 and 25% w/w), and Chronakis (1996) (14 and 20% w/w). For the matrix formed, this "gel-like" behavior reveals permanent intra- and intermolecular rearrangement of effectively elastic bonds among protein molecules (Chronakis, 1996).

Changes in G' and G'' modulus and tan δ values with pH and protein concentration are shown in Figure 6. In all gels, both moduli increased with protein concentration, the raise of the storage modulus (G') being particularly pronounced. This indicates an increase of elasticity, a fact also corroborated by the decrease of tan δ. The decrease in tan δ is related to the development of a large number of contact points among protein molecules or to the formation of a more compact structure at high protein concentrations (Cooney et al., 1993).

Gel Texture. *Effect of pH.* Figure 7 exhibits typical curves recorded during uniaxial compression tests of

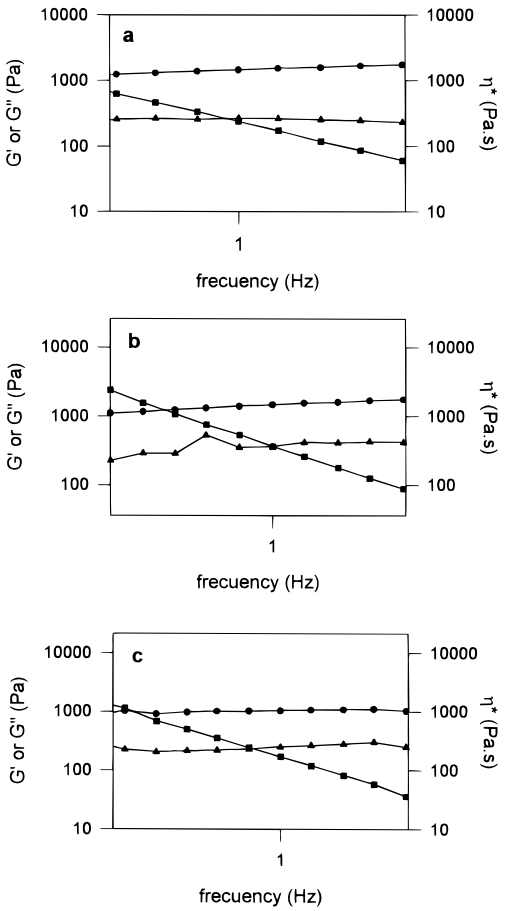

Figure 5. Variation of G' (●), G'' (▲), and η^* (■) as a function of frecuency, of (14% w/w) dispersions of pH 2.75 (a), 3.50 (b), and 8.0 (c).

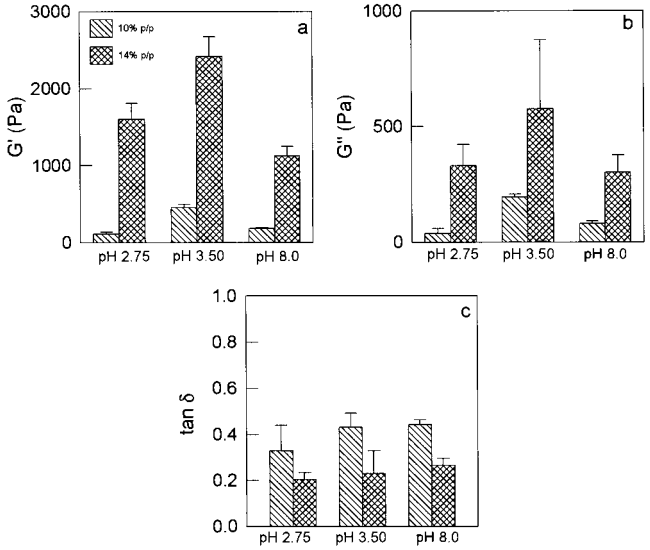

Figure 6. Variation of G' (a), G'' (b), and tan δ (c) as a function of gel protein concentration.

10% w/w protein gels at different pH. Gels at pH below 2.75 and at 8.0 present only one peak, pH 3.00 and 3.25 gels show two totally overlapping peaks while two well-separated peaks are observed at pH 3.50. At extreme pH values, aggregation of proteins is lower due to repulsion of equally charged molecules, leading to ordered and translucent matrix, the texture profile of which presents only one peak. As the pH approaches pI, water−protein interaction decreases and fragile

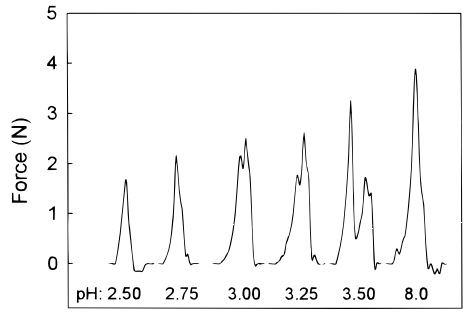

Figure 7. Force-time curves of soy protein gels (10% w/w) prepared in distilled water at different pHs. Compression: 80%. Compression rate: 100 mm/min.

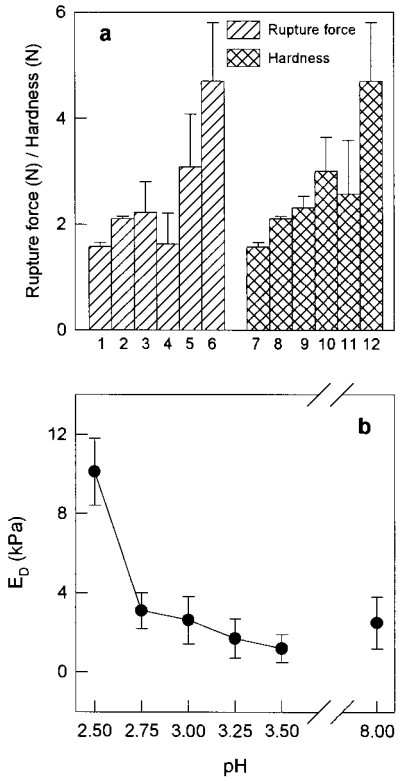

Figure 8. (a) Rupture force (single diagonal lines) and hardness (double cross-diagonal lines) of (10% w/w) gels prepared in distilled water at the pH values: 2.50 (1, 7); 2.75 (2, 8); 3.00 (3, 9); 3.25 (4, 10); 3.50 (5, 11); and 8.0 (6, 12). (b) Modulus of deformability, E_D of 10% w/w gels of several pH, prepared in distilled water.

structure gels are formed. These gels show an initial fracture (first peak) and, after compression progressed, a second rupture (second peak).

In acidic pH gels, the rupture force (F) and hardness (H) are significantly less than those of pH 8.0 gel (Figure 8a). In acidic gels, significant differences ($p < 0.05$) were observed only between samples of extreme pH.

Hardness increases with increasing the number of disulfide bonds in the gel matrix (Furukawa and Ohta, 1982). The behavior of β-lactoglobulin gels at different pH is alike that of soy protein gels. β-Lactoglobulin gels of transparent, fine structure are fragile at low pH and elastic at high pH, while gels presenting an aggregate structure are more likely to fracture above the pI (Stading and Hermansson, 1991).

The deformability modulus (E_D) decreases sharply between pH 2.50 and 2.75, reaching the lower value at pH 3.50 (Figure 8b). The pH 2.50 gel is more resistant

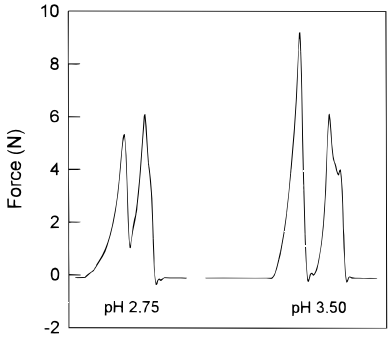

Figure 9. Force-time curves of soy protein gels (14% w/w) of pH 2.75 and 3.50 prepared in distilled water.

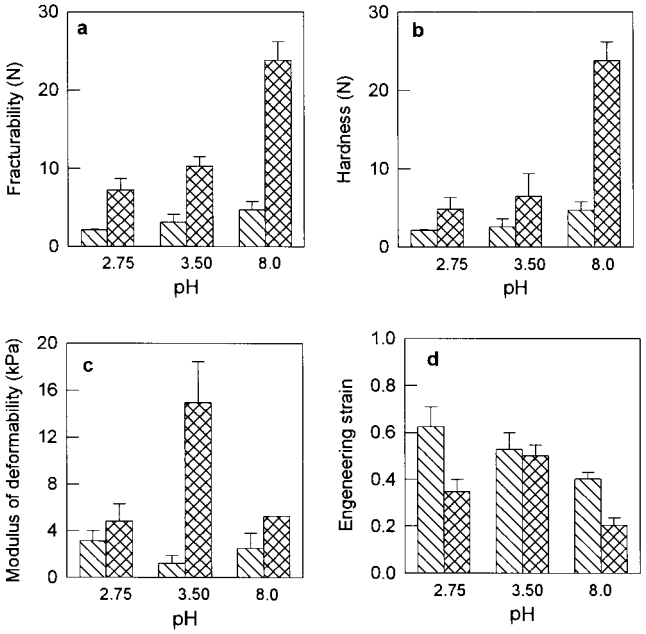

Figure 10. (a) Fracturability, (b) hardness, (c) modulus of deformability, and (d) engeneering strain of different pH and protein concentration gels prepared in distilled water: 10% w/w (single diagonal lines) and 14% w/w (double cross-diagonal lines).

to deformation than the other acidic gels, that property being similar in pH 8.0 and 2.75 gels. These results suggest a low contribution of disulfide bonds, present only in the pH 8.0 gels, to the modulus of elasticity. In this regard, Okamoto et al. (1973) have studied gelatine gels, which present a high content of disulfide bonds, and observed that disulfide bonds contribute slightly to the increase of the elastic modulus. Likewise, in studies on commercial soy proteinate gels, Furukawa and Ohta (1982) have also observed a decrease in the elasticity modulus with the increase of numbers of cleaved disulfide bonds.

Effect of Protein Concentration. The increase in protein concentration from 10 to 14% w/w modifies the texture profile of acidic gels (Figure 9). For pH 2.75, the peak observed in the 10% gel splits in two peaks, revealing a structure with independent fracturability and hardness. In the pH 3.50 gel, there is a clearer separation between the first and second peaks, while at pH 8.0, the gel keeps the texture profile already observed in the 10% gel (profile not shown).

The data plotted in Figure 10 (panels a and b) show an increase of fracturability and hardness for increasing protein concentration. For alkaline pH values, where the sulfhydryl−disulfide interchange is favored, the increase in F and H is more noticeable. This is possibly due to the increase in disulfide bonds as a consequence of stronger protein−protein interaction. Similar results were obtained by Kang et al. (1991) for soy protein isolates and by Nio et al. (1985) and Utsumi et al. (1982) in 7S and 11S globulin gels. The lowest concentration of glycinin required for gel formation is 2.5%, and hardness increases as the globulin concentration and the proportion of acidic high molecular weight subunits present in 11S fraction becomes larger (Nakamura et al., 1984). Hardness of gels prepared from other proteins such as β-lactoglobulin, milk whey protein isolates, and bovine serum albumin presents the same hardness behavior with regard to protein concentration (Lee and Batt, 1993; Lupano et al., 1992).

The deformability modulus (E_D) increases as the protein concentration increases, this raise being more pronounced for the pH 3.50, characterized by a high resistance to deformation before rupture (Figure 10c). In the pH 2.75 and 8.0 gels of higher protein concentration, the engeneering strain (ϵ_f) value is lower (Figure 10d), i.e., they deform less at rupture than at 10% w/w concentration. Gels whose pH is far from the pI behave similarly when protein concentration increases. This fact indicates that noncovalent interactions of equally charged molecules, dominant both at pH 2.75 and at pH 8.0, have a significant effect in gel texture parameters. Close to the pI, where the protein net charge tends to zero, the increase of concentration strengthens protein−protein interactions, so leading to the gel whose structure is more resistant to deformation (very high E_D) and whose ϵ_f is higher than in gels where pH is far from pI.

CONCLUSIONS

From the results described above, it may be concluded that acidic pH dispersions present a viscoelastic behavior. Dispersions of 10% w/w protein concentration behaved as semidiluted macromolecular solutions or pseudogels. At constant frequency (1 Hz), tan δ values increase slightly as pH gets closer to pI, indicating that the more acidic gels are more elastic. The alkaline gel presented the lowest elasticity, possibly because of the rigidity imparted by disulfide ions. The variation in tan δ would be related to the fact that the sulfhydryl−disulfide interchange is not favored at acidic pH and that the protein denaturation percentage due to previous pH treatment is higher at the more acidic pH. The increase of protein concentration from 10 to 14% w/w does not lead to pseudogels but to materials that behave as strong or true gels over the entire frequency range. Nearness of molecules enhance protein−protein interaction so gel elasticity increases. The more acidic gels are more transparent and less firm, showing a closer homogeneous structure with higher water holding capacity than gels whose pH is near pI. Gel microstructure is intimately related with texture; for instance, the more acidic gels present less fracturability and hardness, and higher resistance to deformation. This phenomenon would be caused by the fact that, for pH far from the pI, the water−protein interaction is favored. Besides, it was also observed that all acidic gels are less sensitive to fracture and less hard (softer) than alkaline gels. Fracturability, hardness, and resistance to deformation of gels increase for increasing protein concentration.

LITERATURE CITED

Andersson, Y.; Drake, B.; Granquist, A.; Halldin, L.; Johansson, B.; Pangborn, R. M.; Akesson, C. Fracture force, hardness and brittleness in crisp bread, with a generalized regression analysis approach to instrumental-sensory comparisons. *J. Texture Stud.* **1973**, *4*, 119.

Baird, D. G. Dynamic viscoelastic properties of soy isolate doughs. *J. Texture Stud.* **1981**, *12*, 1−16.

Bertola, N. C.; Califano, A. N.; Bevilacqua, A. E.; Zaritzky, N. E. Textural changes and proteolysis of low-moisture mozzarella cheese frozen under various conditions. *Lebensm.-Wiss. Technol.* **1996**, *29*, 470−474.

Beveridge, T.; Jones, L.; Tung, M. A. Progel and gel formation and reversibility of gelation of whey, soybean, and albumen protein gels. *J. Agric. Food Chem.* **1984**, *32*, 307−313.

Bohlin, L.; Hegg, P.; Ljusberg-Wahren, H. Viscoelastic properties of coagulating milk. *J. Dairy Sci.* **1984**, *76*, 729.

Bourne, M. C. Principles of objetive texture measurement. *Food Texture and Viscosity: Concept and Measurement*; Academic Press: New York, 1982; pp 44−117.

Bowland, E. I.; Foegeding, E. A. Effects of anions on thermally induced whey protein isolate gels. *Food Hydrocolloids* **1995**, *9*, 47−56.

Chronakis, I. S. Network formation and viscoelastic properties of commercial soy protein dispersions: effect of heat treatment, pH and calcium ions. *Food Res. Int.* **1996**, *29*, 123−134.

Chronakis, I. S.; Kasapis, S.; Richardson, R. K.; Doxastakis, G. Characterization of a commercial soy isolate by physical techniques. *J. Texture Stud.* **1995**, *26*, 371−389.

Clark, A. H.; Ross-Murphy, S. B. Structural and mechanical properties of biopolymer gels. *Adv. Polym. Sci.* **1987**, *83*, 60−192.

Cooney, M. J.; Rosenberg, M.; Shoemaker, C. F. Rheological properties of whey protein concentrate gels. *J. Texture Stud.* **1993**, *24*, 325−334.

Cuvelier, G.; Launay, B. Concentration regimes in xanthan gum solutions deduced from flow and viscoelastic properties. *Carbohydr. Polym.* **1986**, *6*, 321−333.

Foegeding, E. A.; Bowland, E. L.; Hardin, C. C. Factors that determine the fracture properties and microstructure of globular protein gels. *Food Hydrocolloids* **1995**, *9*, 237−249.

Furukawa, T.; Ohta, S. Mehcanical and water-holding properties of heat-induced soy protein gels as related to their structural aspects. *J. Texture Stud.* **1982**, *13*, 59−69.

Giboreau, A.; Cuvelier, G.; Launay, B. Rheological behaviour of three biopolymer/water systems, with emphasis on yield stress and viscoelastic properties. *J. Texture Stud.* **1994**, *25*, 119−137.

Haake, information. A general introduction to the theory of forced-oscillation. *Proceedings of the china-japan international conference on rheology*; 1991; pp V92−27E.

Heertje, I. Structure and function of food products: a review. *Food Struct.* **1993**, *12*, 343−364.

Hsieh, Y. L.; Yun, J. J.; Rao, M. A. Rheological properties of mozzarella cheese filled with dairy, egg, soy proteins, and gelatin. *J. Food Sci.* **1993**, *58*, 1001−1004.

Kang, I. J.; Matsumura, Y.; Mori, T.Characterization of texture and mechanical properties of heat-induced soy protein gels. *J. Am. Oil Chem. Soc.* **1991**, *68*, 339−345.

Ker, Y.; Chen, R.; Wu, C. Dynamic viscoelasticity of soybean 11S globulin under shear force at different temperatures. *Biosci. Biotech. Biochem.* **1992**, *56*, 799−800.

Kohyama, K.; Nishinari, K. The effect of glucono-δ-lactone on the gelation time of soybean 11S protein: concentration dependence. *Food Hydrocolloids* **1992**, *6*, 263−274.

Kohyama, K.; Nishinari, K. Rheological studies on the gelation process of soybean 7S and 11S proteins in the presence of glucono-δ-lactone. *J. Agric. Food Chem.* **1993**, *41*, 8−14.

Kohyama, K.; Yoshida, M.; Nishinari, K. Rheological study on gelation of soybean 11S protein by glucono-δ-lactone. *J. Agric. Food Chem.* **1992**, *40*, 740−744.

Lee, S. P.; Batt, C. A. A microscale method for measuring the hardness of heat-induced protein gels. *J. Texture Stud.* **1993**, *24*, 73−79.

Lupano, C. E.; Dumay, E.; Cheftel, J. C. Gelling properties of whey protein isolate: influence of calcium removal by dialysis or diafiltration at acid or neutral pH. *Int. J. Food Sci. Technol.* **1992**, *27*, 615−628.

Nagano, T.; Akasaka, T.; Nishinari, K. Dynamic viscoelastic properties of glycinin and β-conglycinin gels from soybans. *Biopolymers* **1994a**, *34*, 1303−1309.

Nagano, T.; Mori, H.; Nishinari, K. Effect of heating and cooling on the gelation kinetics of 7S globulin from soybeans. *J. Agric. Food Chem.* **1994b**, *42*, 1415−1419.

Nakamura, T.; Utsumi, S.; Kitamura, K.; Harada, K.; Mori, T. Cultivar differences in gelling characteristics of soybean glycinin. *J. Agric. Food Chem.* **1984**, *32*, 647−652.

Nio, N.; Motoki, M.; Takinami, K. Gelation of casein and soybean globulins by transglutaminase. *Agric. Biol. Chem.* **1985**, *49*, 2283−2286.

Nishinari, K.; Kohyama, K.; Zhang, Y.; Kitamura, K.; Sugimoto, T.; Saio, K.; Kawamura, Y. Rheological study on the A_5 subunit on the gelation characteristics of soybean proteins. *Agric. Biol. Chem.* **1991**, *55*, 351−355.

Nussinovitch, A.; Peleg, M.; Normand, M. D. A modified Maxwell and a nonexponential model for the characterization of the stress relaxation of agar and alginate gels. *J. Food Sci.* **1989**, *54*, 1013−1016.

Nussinovitch, A.; Ak, M. M.; Normand, M. D.; Peleg, M. Characterization of gellan gels by uniaxial compression, stress relaxation and creep. *J. Texture Stud.* **1990**, *21*, 37−49.

Okamoto, N.; Wada, T.; Takagi, S.; Yasumatsu, K. Texture of S−S cross-linked gelatin gels. *Agric. Biol. Chem.* **1973**, *37*, 2501−2504.

Owen, S. R.; Tung, M. A.; Paulson, A. T. Thermorheological studies of food polymer dispersions. *J. Food Eng.* **1992**, *16*, 39−53.

Peleg, M. Operational conditions and the stress−strain relationship of solid foods-Theoretical evaluation. *J. Texture Stud.* **1977**, *8*, 283−295.

Puppo, M. C.; Añón, M. C. Soy protein dispersions at acidic pH. Thermal and rheological behavior. *J. Food Sci.* **1998**, submitted for publication.

Puppo, M. C.; Lupano, C. E.; Añón, M. C. Gelation of soybean protein isolates in acidic conditions. Effect of pH and protein concentration. *J. Agric. Food Chem.* **1995**, *43*, 2353−2361.

Ross-Murphy, S. B. Physical gelation of biopolymers. *Food Hydrocolloids* **1987**, *1*, 485−495.

Ross-Murphy, S. B. Rheological characterisation of gels. *J. Texture Stud.* **1995a**, *26*, 391−400.

Ross-Murphy, S. B. Rheology of biopolymer solutions and gels. In *New Physicochemical techniques for the characterization of complex food systems*; Dickinson, E., Ed.; Blackie Academic & Professional: Glasgow, U.K., 1995b; Chapter 6, pp 130−156.

Stading, M.; Hermansson, A. M. Large deformation properties of β-lactoglobulin gel structures. *Food Hydrocolloids* **1991**, 5, 339−352.

Stading, M.; Langton M.; Hermansson, A. M. Inhomogeneous fine-stranded β-lactoglobulin gels. *Food Hydrocolloids* **1992**, *6*, 455−470.

Stading, M.; Langton M.; Hermansson, A. M. Microstructure and rheological behavior of particulate β-lactoglobulin gels. *Food Hydrocolloids* **1993**, *7*, 195−212.

Stamponi, C. R.; Noble, A. C. The influence of fat, acid, and salt on the temporal perception on firmness, saltiness, and sourness of cheese analogues. *J. Texture Stud.* **1991**, *22*, 381−392.

Stamponi, C. R.; Noble, A. C. The influence of fat, acid, and salt on the perception of selected taste and texture atributes of cheese analogues: a scalar study. *J. Texture Stud.* **1992**, *22*, 367−380.

Systat. V5.0 for Windows. SYSTAT, INC., Copyright, 1990.

Utsumi, S.; Kinsella, J. E. Forces involved in soy protein gelation: effects of various reagents on the formation,

hardness and solubility of heat-induced gels made from 7S, 11S, and soy isolate. *J. Food Sci.* **1985a**, *50*, 1278−1282.

Utsumi, S.; Kinsella, J. E. Structure−function relationship in food proteins: subunit interactions in heat-induced gelation of 7S, 11S, and soy isolate. *J. Agric. Food Chem.* **1985b**, *33*, 297−303.

Utsumi, S.; Nakamura, T.; Mori, T. A micro-method for the measurement of gel properties of soybean 11S globulin. *Agric. Biol. Chem.* **1982**, *46*, 1923−1924.

Van Kleef, F. S. M. Thermally induced protein gelation: gelation and rheological characterization of highly concentrated ovalbumin and soy protein gels. *Biopolymers* **1986**, *25*, 31−59.

Van Kleef, F. S. M.; Boskamp, J. V.; Van Den Temple, M. Denaturation of the number of cross-links in a protein gel from its mechanical and swelling properties. *Biopolymers* **1978**, *17*, 225−235.

Wang, C.; Damodaran, S. Thermal gelation of globular proteins: weight-average molecular weight dependence of gel strength. *J. Agric. Food Chem.* **1990**, *38*, 1157−1164.

Wang, C.; Damodaran, S. Thermal gelation of globular proteins: influence of protein conformation on gel strength. *J. Agric. Food Chem.* **1991**, *39*, 433−438.

Yoshida, M.; Kohyama, K.; Nishinari, K. Gelation properties of soymilk and soybean 11S globulin from Japanese-grown soybeans. *Biosci. Biotech. Biochem.* **1992**, *56*, 725−728.

Zheng, B.; Matsumura, Y.; Mori, T. Relationships of molecular forces to rheological and structural properties of legumin gels from broad beans. *Biosci. Biotech. Biochem.* **1993**, *57*, 1257−1260.

Received for review December 23, 1997. Revised manuscript received May 26, 1998. Accepted May 28, 1998. This investigation was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

JF971092N