Structural Properties of Heat-Induced Soy Protein Gels As Affected by Ionic Strength and pH

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

Hydration properties of acidic soy protein gels, prepared with different salt solutions, were studied. The type of bonds that stabilize gel structure and the nature of protein species that make up and stabilize such structure were also investigated. The microstructure of gels was evaluated by scanning electron microscopy (SEM) and water-holding capacity (WHC) assays. The stability and nature of protein fractions of gel matrices were analyzed by solubility measurements and sodium dodecyl sulfate−polyacrylamide gel electrophoresis. The WHC of gels prepared with NaCl and $CaCl_2$ decreased with increasing salt concentration. This fact suggested, as was corroborated by gel SEM, that at high ionic strength a more open matrix was formed. The structure of acidic gels, stabilized by noncovalent bonds, changed with NaCl addition. Both 7S and 11S globulin subunits participated via hydrophobic interactions to the stability of pH 2.75 gels. At pH 3.50 the gel matrix was stabilized by hydrophobic interactions among β-conglycinin subunits, whereas the AB-11S subunit and the AB-11S polymers, linked by disulfide bonds, would be soluble in the matrix interior due to the glycinin fraction that remains native after thermal treatment.

Keywords: *Soy protein gelation; gelation in acidic conditions; heat-induced gelation; salt effect; gel protein structure*

INTRODUCTION

Heat-induced gelation is a very important property for preparing soy-based products such as tofu and yoghurt. The formation of protein networks is considered to be the result of a balance of protein−protein and protein−water interactions and of attraction and repulsion forces occurring between adjacent polypeptide chains (Cheftel et al., 1993). Among attraction forces, one can find hydrophobic interactions (favored at high temperatures), electrostatic forces (calcium bridges), hydrogen bonds (strengthened at low temperatures), and disulfide bonds. The contribution of each bond type depends on the nature of the protein-specific stage in the gelation process as well as on pH and ionic strength of the medium (Utsumi et al., 1984; Utsumi and Kinsella, 1985a,b; Nakamura et al., 1986; Shiga and Nakamura, 1987; Damodaran, 1988; Puppo et al., 1995). The repulsion forces that act especially at pH far from p*I* and protein−water interactions help to keep polypeptide chains separated, thereby favoring the formation of a homogeneous matrix (Heertje, 1993).

Disulfide bond formation on heating makes the gelation process irreversible, as, for instance, in ovalbumin (Van Kleef, 1986) and β-lactoglobulin (Dumay, 1988) gels. By contrast, gelatin gels are stabilized mainly by hydrogen bonds (Ledward, 1986) and melt on heating, and the gelation−melting process can be repeated many times.

Many gels posess hydrated structures (Stading et al., 1992, 1993) and, besides water, are able to retain other food components. Some protein gels retain up to 95% water (w/w); this water is physically immobilized by the protein structure and cannot be released by pressure. The CO and NH groups that are exposed during denaturation become polarized clusters, creating a water multilayer along the polypeptide chain which, on cooling, interact, forming new hydrogen bonds and thus producing the structure required to immobilize free water. Protein network pores can also retain water by capillarity phenomena (Cheftel et al., 1993). One of the more important properties of soy protein gels lies in their capacity to retain water, which depends on pH (Puppo et al., 1995) and on gel ionic strength. This property is closely related to the type of protein structure. Homogeneous and fine structures produce gels with high water retention capacity, whereas gels having nonhomogeneous or particulate structure present a high degree of syneresis. Salt addition favors the formation of coagulate-type gels with low water holding capacity (WHC) (Harwalkar and Kalab, 1985; Heertje and Van Kleef, 1986; Stading and Hermansson, 1991; Foegeding et al., 1995).

The objective of the present work was to determine the hydration properties of acidic gels with different ionic strengths and the type of bonds that stabilize gel structure and to characterize the protein species that make up and/or stabilize such structure.

MATERIALS AND METHODS

Soy Protein Isolate. Soy protein isolates were prepared from defatted low-heat soy meal produced by Santista Alimentos S.A. (Brazil). Soy flour was dispersed in distilled water (1:10, w/w). The dispersion was adjusted to pH 8.0 with 2 N NaOH, stirred at room temperature for 2 h, and centrifuged at 13300*g* for 20 min at 15 °C. The supernatant was then

* Author to whom correspondence should be addressed (telephone +54-21-254853; e-mail mca@nahuel.biol.unlp.edu.ar).

S0021-8561(98)00006-5 CCC: $15.00
Published on Web 08/13/1998

adjusted to pH 4.5 with 2 N HCl and centrifuged at 3300*g* for 20 min. The pellet was resuspended with distilled water, adjusted to pH 3.25 or 8.0 with 2 N HCl or 2 N NaOH to obtain the acidic and the basic isolates, respectively, and then freeze-dried (Puppo et al., 1995). The acidic dispersions of different pH values were obtained from the pH 3.25 isolate by adjusting the pH with 1 N HCl or 1 N NaOH. All chemicals used were of analytical grade.

Preparation of Gels. The minimum protein concentration required for heat-induced gelation (90 °C, 30 min) from the isolates was 7% w/w. Dispersions in distilled water, NaCl (0.05−2.0 M) and $CaCl_2$ (0.1 and 0.2 M) salt solutions (10% protein, w/w) from pH 3.25 soy protein isolate, were prepared and adjusted to pH 2.75 and 3.50. Dispersions (10% protein, w/w) from the pH 8.0 soy protein isolate were also prepared in solutions of 0.1, 0.25, and 2.0 M NaCl concentration. In each case the pH was adjusted with 1 N HCl or 1 N NaOH. Dispersions were partially deaerated by centrifugation at 1000*g* for 1 min at 15 °C, carefully resuspended with a glass rod, and placed in glass tubes (2.2 cm i.d. × 6 cm height) with tightly closed stoppers. Gelation was then carried out using the method described by Puppo et al. (1995), heating the glass tubes in a water bath at 90 °C for 30 min and then cooling immediately in a water bath at 15 °C to ensure complete gelation. Gel samples were kept at 4 °C for 48 h before analysis.

Scanning Electron Microscopy (SEM). SEM assays were conducted on gels of pH 2.75, 3.50, and 8.0 (10% w/w) prepared with NaCl (0.1−2.0 M) and $CaCl_2$ (0.1 and 0.2 M) solutions obtained according to the method described above. Gel samples were immersed in 2.5% glutaraldehyde with 0.1% w/v ruthenium red and 0.025 M KCl for 72 h and washed several times with 0.025 M KCl followed by 2% w/v OsO_4 for 2 h at 4 °C. The samples were then rinsed for 1 h in distilled water before being dehydrated in a grade acetone series, 25, 50, 70, 90, and 3 × 100% v/v and dried at the critical point. Each dried sample was mounted on a bronze stub and coated with gold, the specimens being observed with a JEOL 35 CF scanning electron microscope at an acceleration voltage of 5 kV.

WHC of Gels. Gel (0.3−1.3 g), equilibrated at room temperature, was placed on a nylon plain membrane (5.0 mm pores, Micronsep) maintained in the middle position of a 50 mL centrifuge tube. Water loss was determined by weighing before and after centrifugation at 120 g for 5 min at 15 °C. WHC was expressed as percent of the initial water remaining in the gel after centrifugation. Each value is the mean (± standard deviation) of three determinations.

Solubility of Gels. Samples were dispersed in distilled water (DW), in a pH 8.0 buffer (0.086 M Tris/0.09 M glycine/4 mM Na_2EDTA) (B), or in the same buffer containing 8 M urea and 0.5% sodium dodecyl sulfate (SDS) (BSU) (Shimada and Cheftel, 1988). Samples (1.1% protein, w/v) were homogenized with a Virtis 23 homogenizer (The Virtis Co., Inc., Gardiner, NY) at room temperature for 30 s and then centrifuged at 18800*g* for 15 min at 20 °C. Protein solubility was determined from supernatants and expressed as 100 × protein content in the supernatant/total protein content. Three independent extractions were carried out with each solvent. Average values (± standard deviation) are reported. Protein concentration was determined spectrophotometrically at 280 nm with an apparent $E^{1\%}_{1cm}$ of 12.04.

The data were statistically analyzed by a two-way model of analysis of variance (ANOVA). Sources of variation were the salt (NaCl or $CaCl_2$) concentration and the extraction media (DW, B, and BSU). The significance of differences among means of the several treatments was determined by Tukey's test at $p < 0.05$.

Electrophoresis (SDS−PAGE). One- and two-dimensional sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) was performed according to the method of Laemmli (1970) as modified by Petruccelli and Añón (1994) using in both cases a linear gradient separating gel (4−15% in polyacrylamide). A continuous dissociating buffer system was used, containing 0.375 M Tris-HCl, pH 8.8, and 0.1% SDS for the separating gel and 0.025 M Tris-HCl, 0.192 M glycine, and 0.1% SDS, pH 8.3, for the run buffer. Prior to the second electrophoresis, each first-dimension slab gel portion was treated with 10 volumes of SDS buffer composed of 62.5 mM Tris-HCl, pH 6.8, 1% SDS, 0.2 M β-mercaptoethanol (ME), and 20% sucrose for 30 min at 55 °C with two changes of solution. The treated gel was placed on the top of the second-dimension SDS slab gel, and the electrophoresis was carried out at a constant voltage of 200 V. Protein solutions were diluted with an equal volume of a pH 6.8 buffer (0.125 M Tris-HCl, 0.1% SDS, 40% v/v glycerol, 0.05% bromophenol blue), with or without 5% ME, and then heated in a boiling water bath for 5 min. Low molecular weight markers (Pharmacia calibration kit) used included phosphorylase *b* (94 kDa), albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa), and α-lactalbumin (14.4 kDa).

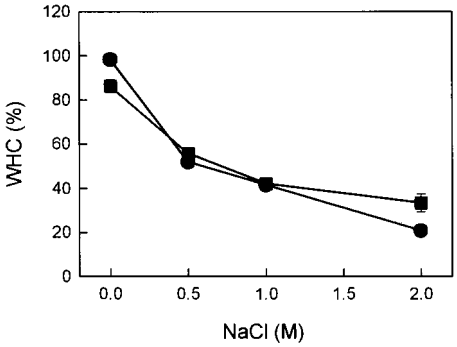

Figure 1. WHC of heat-induced (90 °C, 30 min) soy protein gels (10% w/w) of pH 2.75 (●) and pH 3.50 (■), prepared with solutions of different NaCl concentrations.

RESULTS AND DISCUSSION

Gels Prepared with NaCl Solutions. *WHC and SEM.* For gels at pH 2.75 and 3.50, Figure 1 shows that WHC decreased with increasing gel ionic strength. The WHC decrease (of ∼50%) was more pronounced at low NaCl concentrations (<0.5 M). Above 0.5 M, sodium chloride leads to gels with an open structure and low water retention at both pH values (2.75 and 3.50). This behavior could be due to the stabilizing effect of NaCl on the thermally induced changes in soy globulin structure (Utsumi and Kinsella, 1985b). The sodium chloride is known to stabilize protein structure by stabilizing hydrophobic interactions (Babajimopoulos et al., 1983). Opacity increased very markedly in acidic gels after addition of 0.5 M NaCl, whereas, for NaCl concentrations above 0.5 M, the gels of low WHC exhibited the same opacity but developed a more open and fragile structure (Puppo and Añón, 1998a) as ionic strength increased.

The gels prepared with distilled water of pH 3.50 had a microstructure more aggregated and less homogeneous than those at pH 2.75 and 8.0. At pH far from the p*I* protein−protein interactions decrease and the electrostatic repulsion increases (Puppo et al., 1995). Figure 2 shows micrographs of pH 2.75 gels prepared with NaCl solutions of several concentrations. The less saline gels (0.1 and 0.25 M NaCl) had a more compact structure, whereas those prepared with 0.5 M NaCl exhibited a more open and less homogeneous structure owing to protein aggregation observed at high ionic strengths. The addition of 0.1 M NaCl produced pH 3.50 gels with tight structure, whereas, for higher salt contents (0.25−2.0 M), gels with particulate structure were obtained. The most open structure was observed after addition of 2.0 M NaCl (Figure 3).

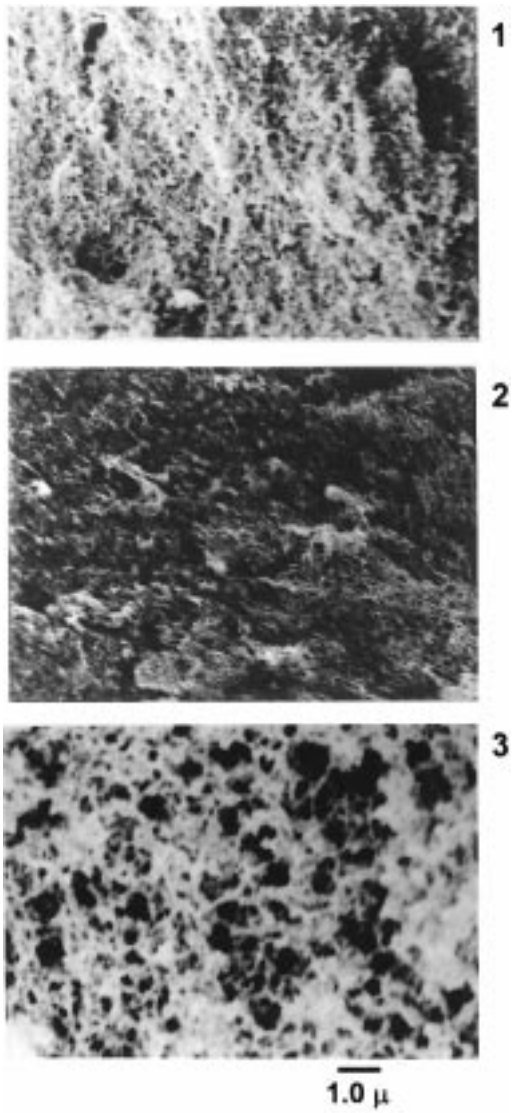

Figure 2. SEM of heat-induced (90 °C, 30 min) gels of pH 2.75 (10% w/w) prepared with 0.1 M (1), 0.25 M (2), and 0.5 M NaCl (3). Magnification: 6000×.

The formation of fine or particulate gels depends on pH and ionic strength. Aggregated polypeptidic chains are formed close to the protein p*I* and/or at high ionic strengths, whereas far from the p*I* (very low or high pH), fine polypeptidic chains are formed (Harwalkar and Kalab, 1985; Van Kleef, 1986; Heertje and Van Kleef, 1986; Stading and Hermansson, 1991). In our work, pH 3.50 gels prepared with NaCl concentrations would belong to the particulate gel category. The neutral salts that favor salting-out at concentrations above 0.15 M and near the p*I* such as NaCl stabilize the protein by reinforcing hydrophobic interactions among molecules and decreasing its solubility (Damodaran and Kinsella, 1982; Foegeding et al., 1995). Other proteins (milk whey proteins) also form opaque, coagulate-type gels of low hardness with a high degree of syneresis at NaCl concentrations above 0.2 M (McClements et al., 1993).

Protein Solubility of Gels. Figure 4 shows the solubility of pH 2.75 and 3.50 gels in several extraction media (DW, B, and BSU) as a function of the NaCl concentration added to the gel. In the gels at pH 2.75, solubility in DW decreased for increasing NaCl concentration in the gel (Figure 4a) and then stabilized above 1.0 M ($p < 0.05$). In contrast, solubility in the B medium slightly

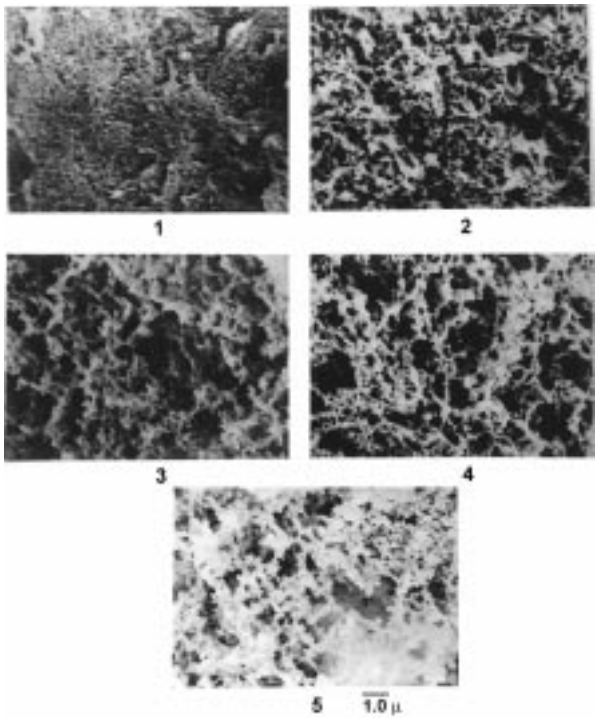

Figure 3. SEM of heat-induced (90 °C, 30 min) gels of pH 3.50 (10% w/w) prepared with 0.1 M (1), 0.25 M (2), 0.5 M (3), 1.0 M (4), and 2.0 M NaCl (5). Magnification: 6000× (figure is reproduced here at 75% of the original).

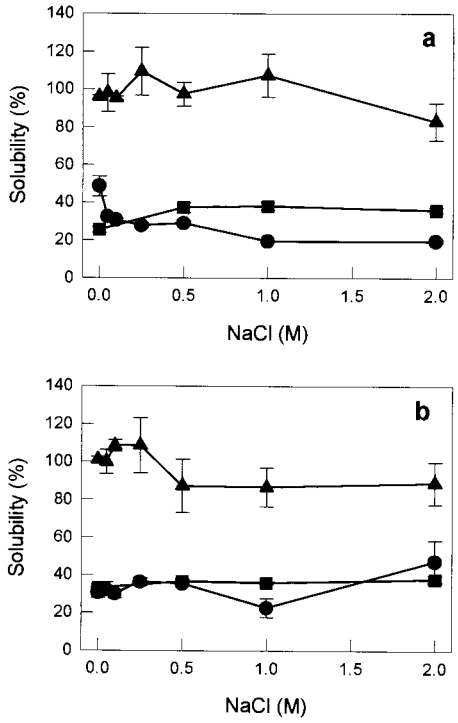

Figure 4. Protein solubility of heat-induced (90°, 30 min) gels (10% w/w) of pH 2.75 (a) and pH 3.50 (b) in DW (●), B (■), and BSU (▲) extraction media versus gel NaCl concentration: (a) $\Delta_{0.05} = 14.99$; (b) $\Delta_{0.05} = 20.67$.

increased for NaCl contents between 0 and 0.5 M and remained constant above 0.5 M at a value slightly higher than that in DW ($p < 0.05$). Hermansson (1978) suggested that there is a limiting ionic strength ($\mu = 0.1$) above which the salting-out effects appear. Addition of salt ($\mu > 0.5$) neutralizes the protein electrostatically and stabilizes it, in addition to favoring the

hydrophobic interactions that insolubilize the protein in DW and increase its solubility in the pH 8.0 buffer (B medium). Solubility in BSU was almost 100% with no significant difference among the several gels ($p < 0.05$).

For pH 3.50 gels, DW solubility remained constant up to 1.0 M (≈30%) and increased when gels were prepared with 2.0 M NaCl ($p < 0.05$) (Figure 4b). Close to the p*I* and for very high ionic strengths (2.0 M NaCl), a high solubilization of the protein was observed in DW medium, possibly because of the thermal stabilization that avoids the denaturation and aggregation required for gelation. Hermansson (1978) has indicated that, at $\mu < 0.1-0.2$, 7S and 11S fractions exist at pH 7.6 in the dissociated form, thus favoring the thermal aggregation, which reaches a maximum for $\mu = 0.2$. Above that salt concentration, such aggregation is inhibited with an increase in protein solubility. The bond of cations and/or anions, coming from salts, to proteins greatly affects protein solubility, these attachments being influenced by the protein secondary and tertiary structures (Kumosinski, 1990). For neutral pH, solubility of both native and denatured soy protein isolates decreases up to a minimum after salt addition and then increases until reaching a constant value for salt concentrations of ~1.0 M (Kumosinski, 1990). The salt so added would enlarge the hydrophobic surface area of the protein, thus promoting autoassociation of molecules followed by salting-in effect. This feature is caused by the increase of protein dipolar moment due to a nonspecific solvation effect (Kumosinski, 1990). This effect is magnified in this case by the thermal treatment.

With regard to B and BSU media, the solubility of gels having pH values close to the p*I* does not vary with the NaCl concentration of the gel, solubility values being 35% in B and 100% in BSU media ($p < 0.05$) (Figure 4b). For ionic strengths < 0.5 and pH close to the p*I*, protein aggregation is dominant and makes gel (in the absence of denaturing agent) solubility independent of the extraction buffer. For $\mu = 2.0$, however, there is a protein fraction that is highly soluble in DW. As a consequence of the acidic treatment applied before the heating used in gelation (90 °C, 30 min), the protein becomes partially denatured regardless of the presence of 2.0 M NaCl. In dispersions prepared in DW, the native protein fraction denatures by thermal treatment action, whereas with 2.0 M NaCl both globulins become thermally stable, with heat denaturation of only β-conglycinin during gelation (Puppo and Añón, 1998b).

SDS−PAGE of Gel Extracts. For gels prepared in 2.0 M NaCl, Figure 5 shows two-dimensional electrophoretic diagrams and the corresponding one-dimensional profiles of the fractions that are soluble in BSU and DW media. Addition of 2.0 M NaCl to the pH 8.0 protein dispersion prevented gelation under the conditions of the heat treatment (90 °C, 30 min). Gelation did not occur because the denaturation temperatures of 7S and 11S are, for that ionic strength, above 120 °C (Puppo and Añón, 1998b). As the gel is not formed, all of the subunits that make up glycinin and β-conglycinin can then be extracted from the thermally treated dispersion in DW and BSU (Figure 5a,b). In BSU, the electrophoretic profile (one- and two-dimensional) is independent of acidic pH, a fact denoting dominance of noncovalent bonds in the gel matrix stabilization (Figure 5c,e).

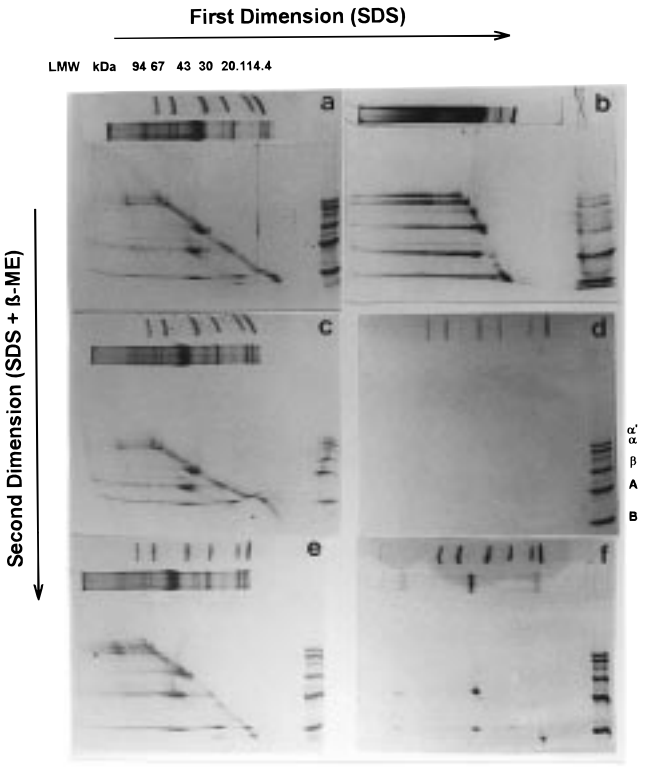

Figure 5. Bidimensional SDS−PAGE of the BSU (a, c, e) and DW (b, d, f) soluble fractions of heat-induced (90 °C, 30 min) gels (10% w/w), prepared with 2.0 M NaCl solution: gels of pH 8 (a, b), pH 2.75 (c, d), and pH 3.50 (e, f).

The two-dimensional profiles of the BSU-soluble fraction were similar in acidic gels (pH 2.75 and 3.50) prepared in 2.0 M NaCl (Figure 5c,e). The profiles show high molecular weight soluble aggregates, consisting mainly of α′- and α-7S and α′-, α-, and β-7S subunits. The AB subunit can also be observed, along with the A- and B-11S polypeptide, the aggregated B polypeptide, and small amounts of glycinin-free A polypeptide. A peptide having a molecular mass below 14 kDa was also detected. Concerning the DW-soluble fraction, the two-dimensional profiles of acidic gels prepared with 2.0 M NaCl were different (Figure 5d,f), and these profiles coincide with solubility values observed in Figure 4. For both gels, the amounts of protein placed in the first dimension were the same. In the pH 2.75 gels, no protein species were observed in the (one- and two-dimensional) electrophoretic profiles (Figure 5d), possibly because the amount of protein extracted in DW at that ionic strength is small (20%). The electrophoretic profile in the pH 3.50 gel presented high molecular weight soluble aggregates formed by A- and B-11S polypeptides, the AB-11S subunit, peptides having molecular mass below 14 kDa, and a very small proportion of the free B polypeptide (Figure 5f). The β-7S subunit and the monomeric A-11S disappear from the profile, while the amount of low molecular weight (<14 kDa) peptides decreases (Figure 5f). A high ionic strength shifts the endotherms of the 11S fraction (pH 3.50) to denaturation temperatures higher than the gelation value (90 °C), thereby preventing thermal denaturation (Puppo and Añón, 1998b). The gel structure stabilized mainly by the 7S fraction would contain 11S globulin solubilized in the matrix interior. In pH 3.50 gels prepared with 2.0 M NaCl, the fact that the 11S fraction solubilizes in DW coincides with the gel solubility increase at such ionic strength.

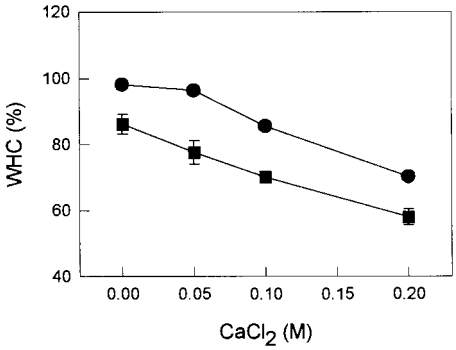

Figure 6. WHC of heat-induced (90 °C, 30 min) soy protein gels (10% w/w) of pH 2.75 (●) and pH 3.50 (■), prepared with different $CaCl_2$ concentration solutions.

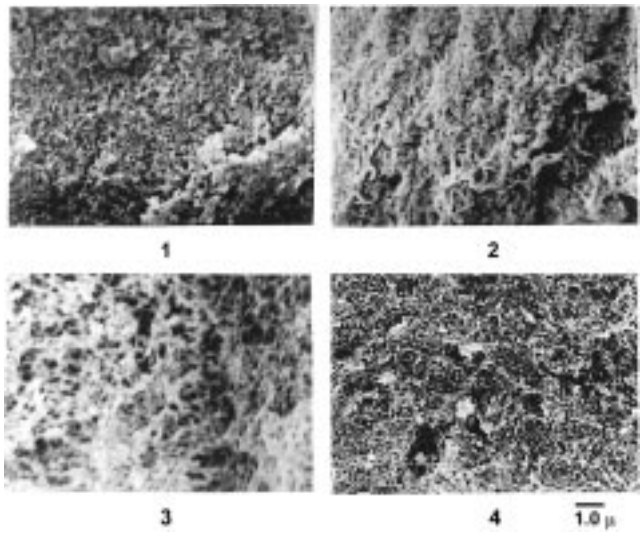

Figure 7. SEM of heat-induced (90 °C, 30 min) gels (10% w/w) of pH 2.75 (1, 2) and pH 3.50 (3, 4) prepared with 0.1 M (1, 3) and 0.2 M (2, 4) $CaCl_2$ solutions. Magnification: 6000× (figure is reproduced here at 72% of the original).

Gels Prepared with $CaCl_2$ Solutions. *WHC and SEM.* The WHC of gels prepared at acidic pH decreased when a divalent salt such as $CaCl_2$ was added (Figure 6). As the ionic strength increased from 0 to 0.5 (0.2 M $CaCl_2$), the WHC decreased for both gels by ~30%. For the same variation in ionic strength, the WHC was modified less than it was by NaCl. In the entire ionic strength range covered, the gels at pH 2.75 had higher WHC than the pH 3.50 gels (Figure 6). Unlike what was observed in the presence of NaCl, acidic gels presented a finer and tighter structure when $CaCl_2$ concentration increased from 0.1 ($\mu = 0.25$) to 0.2 M ($\mu = 0.5$) (Figure 7). In pH 2.75 gels, a high $CaCl_2$ concentration promoted a gel matrix formed by chains of protein threads (Figure 7). At acidic pH, the Ca^{2+} ion competes with the H^+ for the same binding centers (Kroll, 1984), so that Ca^{2+} would not establish bridges with the protein as at alkaline pH. Ca^{2+} could interact with water, thus modifying the aqueous surroundings of the protein, increasing protein aggregation and decreasing WHC. Ions affect protein conformation by electrostatic interactions with the charged groups and the protein polar groups or by hydrophobic interactions between protein molecules (Damodaran and Kinsella, 1982). In the latter case, the salt-induced change in water structure (destabilization of hydrogen bonds) caused by ion−dipole interaction may alter the degree of hydration as well as the orientation of water molecules around the nonpolar residues of polypeptide chains (Damodaran and Kinsella, 1982).

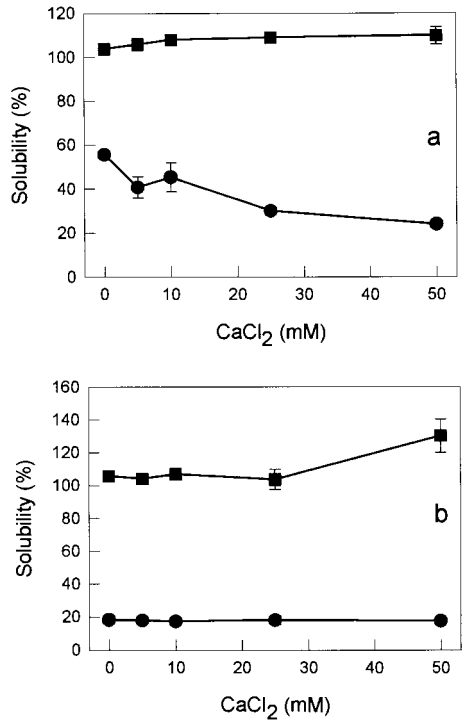

Figure 8. Protein solubility of heat-induced (90°, 30 min) gels (10% w/w) of pH 2.75 (a) and pH 3.50 (b) in DW (●) and BSU (■) extraction media versus gel $CaCl_2$ concentration: (a) $\Delta_{0.05} = 8.40$; (b) $\Delta_{0.05} = 8.52$.

$CaCl_2$, a divalent salt, is an exception in the salting-out effect of the Hofmeister series. Combined with β-lactoglobulin, this salt forms particulate gels at an ionic strength <0.15 M (Foegeding et al., 1995; Bowland and Foegeding, 1995).

Protein Solubility of Gels. For increasing $CaCl_2$ concentrations in pH 2.75 gels, solubility decreased in DW and remained constant in BSU ($p < 0.05$) (Figure 8a). Gels prepared at pH 3.50 showed a different behavior in relation to $CaCl_2$ addition: solubility in DW did not change (20%) and was constant (100%) in BSU ($p < 0.05$) (Figure 8b). Values that increase above 100% would be caused by an artifact of UV determination of soluble protein as a consequence of Ca−protein interaction.

SDS−PAGE of Gel Extracts. For the purpose of determining the protein species making up the structure of gels prepared with $CaCl_2$, the DW soluble (S) and insoluble (I) fractions of the gels were analyzed by electrophoresis (Figures 9 and 10). At alkaline pH, the protein precipitates at very low $CaCl_2$ concentrations (10 mM) (Lee and Rha, 1977); therefore, the DW-soluble fraction of the pH 8.0 gel did not present visible bands after $CaCl_2$ was added to the gel (data not shown). Figure 9 shows the electrophoretic profiles of the DW-insoluble fraction of pH 8.0 gels prepared with $CaCl_2$.

In the presence of $CaCl_2$, the one-dimensional electrophoretic profile developed by the alkaline gel was different from that of the gel prepared in DW (Puppo et al., 1995). Band intensity increased for increasing amounts of $CaCl_2$ (Figure 9a, lanes 3 and 4); therefore, the precipitate contained more protein after salt addition. We observed a wide band representing soluble aggregates of MW 185 (I), a continuum of soluble aggregates of molecular masses ranging from 185 to 55

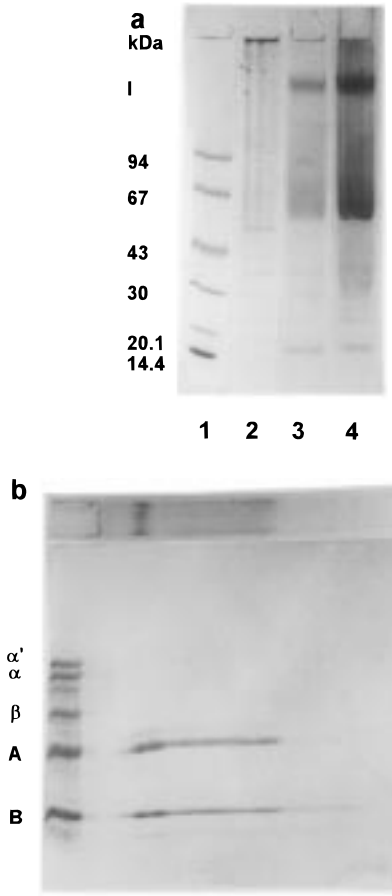

Figure 9. Unidimensional SDS−PAGE of DW insoluble fraction (a) of the pH 8.0 gel (10% w/w) prepared with 0 M (2), 0.1 M (3), and 0.2 M (4) $CaCl_2$ solutions; bidimensional SDS−PAGE of DW insoluble fraction (b) of the pH 8.0 gel prepared with 0.2 M $CaCl_2$; low molecular mass markers (1).

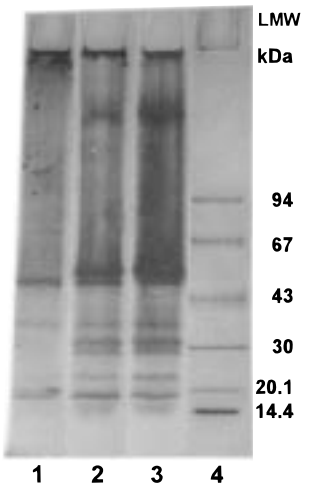

Figure 10. Unidimensional SDS−PAGE of DW insoluble fraction, containing urea and SDS, of the pH 8.0 gel prepared with 0 M (1), 0.05 M (2), and 0.2 M (3) $CaCl_2$ solutions; low molecular mass markers (4).

kDa, and the AB-11S subunit (55 kDa), as well as a 16.5 kDa peptide. This low molecular weight peptide would have been purified together with soy globulins during isolate preparation. From the bidimensional profile (Figure 9b) it can be observed that all-soluble aggregates consist of A- and B-11S polypeptide linked by disulfide bonds.

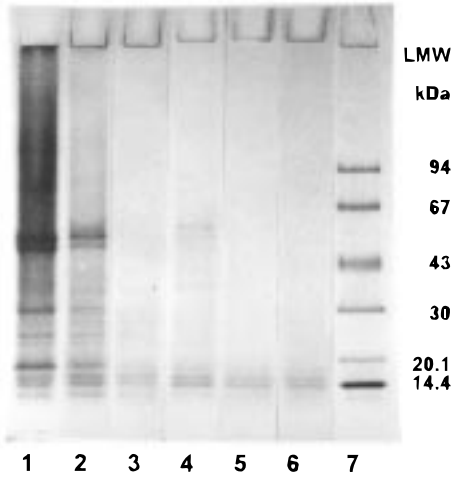

Figure 11. Unidimensional SDS−PAGE of DW soluble fraction of the pH 2.75 (1−3) and pH 3.50 (4−6) gels prepared with 0 M (1, 4), 0.05 M (2, 5), and 0.2 M (3, 6) $CaCl_2$ solutions; low molecular mass markers (7).

When the DW-insoluble fraction was added with the pH 8.0 containing urea and SDS buffer (BSU), we obtained a gel that, in the absence of $CaCl_2$ (Figure 10, lane 1), gave a profile showing dimeric forms of the B- and B-11S polypeptide. In the presence of $CaCl_2$ the profiles (Figure 10, lanes 2 and 3) showed high molecular weight soluble aggregates that could not penetrate the gel and 185 kDa soluble aggregates formed by A- and B-11S polypeptides, as well as α', α, and β subunits of the 7S fraction (the last is the most intense). Also apparent were the AB-11S subunit band, those of polypeptides A and B, and that of the dimeric form B of the 11S fraction. The β-conglycinin, which did not appear in the electrophoretic profile of Figure 9 (lanes 3 and 4), would be insolubilized in the sample buffer. Urea and SDS would induce rupture of noncovalent bonds between subunits, thus solubilizing part of the globulin. The other part would remain in the high molecular weight aggregates which did not penetrate the gel.

The electrophoretic behavior of acidic pH gels was different. In this regard, the DW-soluble fraction (S) of pH 2.75 and 3.50 gels prepared with different amounts of $CaCl_2$ presented one-dimensional profiles (Figure 11) where the band intensity decreased for increasing salt contents; this fact confirms the increase of protein aggregation for increasing ionic strengths. At a $CaCl_2$ concentration of 0.2 M, only a large number of low molecular weight (<20 kDa) peptides was observed. The profiles of the DW-insoluble fraction (I) neither changed with $CaCl_2$ content in the gel nor showed low molecular weight peptides (data not shown). In acidic gels, denaturing agents intensified only the bands of those peptides already existing in the DW-insoluble fraction (data not shown).

Conclusions. Under the same ionic strength conditions, NaCl and $CaCl_2$ induce the same variation in WHC and solubility of acidic pH soy protein gels in the different extraction media.

The structure of acidic gels is stabilized by noncovalent bonds, and these bonds are destabilized by the presence of denaturing agents. By contrast, alkaline gel structure is partially stabilized by disufide bonds between the AB-11S subunit and A and B-11S polypeptides (Puppo et al., 1995). NaCl would contribute to stabilize the structure of the pH 2.75 gel, where all 7S

and 11S globulin subunits would participate by means of hydrophobic interactions. In the presence of salt and at pH 3.50, where a glycinin fraction remains in the native state after heating, protein structure would be stabilized by hydrophobic interactions among 7S globulin subunits, where the AB-11S subunit and the AB-11S polymers (trimeric forms) linked by disulfide bridges (Wolf and Nielsen, 1996) would exist as soluble peptides in matrix interstices.

In the presence of $CaCl_2$, the structure of a pH 8.0 gel would be stabilized by the 7S fraction and mainly by glycinin AB subunit polymers of various molecular weights. In turn, the matrix of acidic gels would be stabilized by glycinin and β-conglycinin, the interstices of which would contain low molecular weight peptides in soluble form.

Knowledge of the structural properties of these gels could expand the use of soy proteins in the formulation of acid foods.

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Received for review January 5, 1998. Revised manuscript received May 11, 1998. Accepted July 3, 1998. This investigation was supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina. M.C.P. is a Research Fellow of the Comisión Nacional de Investigaciones Científicas y Técnicas (CONICET), and M.C.A. is a Member of the Researcher Career of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

JF980006W