Gelation of Soybean Protein Isolates in Acidic Conditions. Effect of pH and Protein Concentration

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Heat-induced gels were obtained from acidic and pH 8.0 soybean protein isolates, at different pH values and protein concentrations. The isolates were characterized through solubility assays in different extraction media, and bidimensional SDS–PAGE, scanning electron microscopy and water-holding capacity were performed to analyze gels. Results indicate that acidic gels became more aggregated when the pH approached the p*I*. Gels obtained at acidic pH were different from those obtained at pH 8.0, the latter having more interchain disulfide bonds and different protein species involved in the maintenance of the structure.

Keywords: *Gelation of soybean proteins; soybean proteins; gelation in acidic conditions*

INTRODUCTION

The two major reserve soybean proteins are the 7S β-conglycinin and 11S glycinin globulins. These proteins play an important role in several food systems because of their high nutritional value and their functional properties. The 7S globulin is a trimeric glycoprotein (141 000–170 000 Da) composed of three subunits, α (57 000 Da), α′ (58 000 Da), and β (42 000 Da), associated by hydrophobic interactions (Thanh and Shibasaki, 1977). The 11S globulin consists of two apposed hexagonal rings each containing three hydrophobically associated subunits consisting of pairs of disulfide-linked acidic (35 000–37 000 Da) and basic (20 000 Da) polypeptides (Moreira et al., 1979; Nielsen, 1985). These proteins may dissociate and associate in different ways and form gels upon heating (Kang et al., 1991; German et al., 1982; Damodaran and Kinsella, 1982). The capacity of gels to retain water, lipids, sugars, flavors, and other ingredients is very useful to the development of new products. Gel characteristics are strongly affected by heating time, temperature, ions, and pH; thus, different gels can be obtained by varying the heating conditions (Bau et al., 1985; Mori et al., 1982, 1986; Utsumi and Kinsella, 1985a,b). Most of the studies on gelling and other heat-induced interactions between soybean proteins have been performed at pH 7.0–8.0 (Utsumi et al., 1984; Utsumi and Kinsella, 1985a,b; Nakamura et al., 1986; Shiga and Nakamura, 1987; Damodaran, 1988). In Tris, pH 8.0, and in the presence of β-mercaptoethanol (ME) (protein concentration 0.5%) heating causes dissociation of both 7S and 11S globulins; the dissociated subunits subsequently interact with each other, forming soluble macrocomplexes of MW over 1 million. These macrocomplexes contained predominantly the basic subunits of 11S globulin and the β-subunits of 7S globulin (Utsumi et al., 1984). Studies performed in gels obtained by heating soybean protein solutions (12–15% w/v, Tris, pH 8.0) in the presence of different reagents (NaCl, NaSCN, propylene glycol, ME) showed that the possible molecular forces involved in the formation of soy isolate gels are hydrogen bonds and hydrophobic interactions, whereas in the gel maintenance the possible forces are disulfide and hydrogen bonds (Utsumi and Kinsella, 1985a,b).

Bau et al. (1985) observed important differences in the viscosity of soy protein isolate dispersions heated at different pH values. Also, some studies were performed on the gelling properties of milk whey proteins at acidic pH (Stading and Hermansson, 1991; Lupano et al., 1992). However, little is known concerning the gelling properties of soy protein isolates at acidic pH. These gels could be utilized in the formulation of acid foods. Different properties are expected in acid gels because forces involved in the maintenance of gel structure may be different at acid pH. The objective of this work was to analyze the gelling properties of a soy protein isolate obtained at acid pH. Gels were prepared in distilled water. As we planned, for future work, to include these gels in food formulations, no chemical additives were utilized in their preparation.

MATERIALS AND METHODS

Soy Protein Isolate. Soybean protein isolate (SPI) was prepared from defatted low-heat soybean meal produced by Sanbra 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 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 SPI (SPI 1) and the basic SPI (SPI 2), respectively, and then freeze-dried. The 7S/11S ratio of SPI was 0.85 ± 0.05, calculated from a densitogram obtained by means of a TLC scanning CS-910 double-wavelength Shimadzu spectrodensitometer. All chemicals used were of analytical grade.

Gelation of SPI Dispersions. Aqueous dispersions (10% protein, w/w) of SPI 1 and SPI 2 were prepared. Those of SPI 1 were adjusted to pH 2.50, 2.75, 3.00, 3.25, or 3.50 with 1 N HCl or 1 N NaOH. In some cases (pH 2.75 and 3.50) dispersions were performed at different protein concentrations (8, 10, 12, and 14% w/w). Dispersions were partially deaereated by centrifugation at 1000*g* for 1 min at 15 °C (Xiong and Kinsella, 1990), carefully resuspended with a glass rod, and placed in glass tubes (2.2 cm i.d. × 6 cm height) with

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tightly closed stoppers. Gelation was then carried out by heating the glass tubes in a water bath at 90 °C for 30 min (Damodaran, 1988) and then cooling immediately in a water bath at 15 °C. Gel samples were kept at 4 °C for 24–48 h before analysis.

Water-Holding Capacity (WHC) of Gels. Gel (0.3–1.3 g), equilibrated at room temperature, was placed on a nylon plain membrane (5.0 μm 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 (Quéguiner et al., 1989). WHC was expressed as percent of the initial water remaining in the gel after centrifugation. Each value is the mean (± standard deviation) of at least four determinations.

Solubility of SPI Powders and 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.

Electrophoresis (SDS–PAGE). One- and two-dimensional sodium dodecyl sulfate–polyacrylamide gel electrophoreses (SDS–PAGE) were 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 (0.125M 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 MW markers (Pharmacia calibration kit) used included phosphorylase *b* (94 000), albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), trypsin inhibitor (20 100), and α-lactalbumin (14 400).

Scanning Electron Microscopy. 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.

RESULTS AND DISCUSSION

Soy Protein Isolates. Protein contents (N × 6.25) were 82.4 ± 0.4 and 75.7 ± 0.8% and moisture contents were 10.2 ± 0.5 and 12.7 ± 0.0% for SPI 1 and SPI 2, respectively.

Figure 1 shows the protein solubilities of SPI 1 and SPI 2 in distilled water (DW), pH 8 buffer (B), and pH 8 buffer containing urea and SDS (BSU). The protein solubility of SPI 2 in DW or BSU was about 100%, whereas in B it was 80%. The isoelectric pH (p*I*) of soy proteins is about 4.5; thus, these proteins have negative

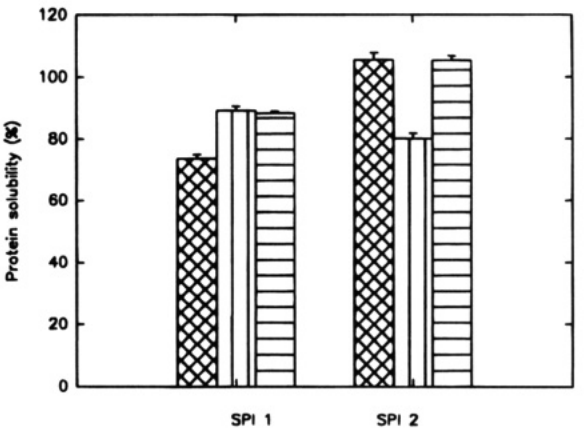

Figure 1. Protein solubility of acidic (SPI 1) and basic (SPI 2) soybean protein isolates (0.1% protein, w/v). Extraction solutions: (cross-hatched bar) distilled water; (vertically striped bar) buffer pH 8; (horizontally striped bar) buffer pH 8–SDS–urea. The bars show standard deviation.

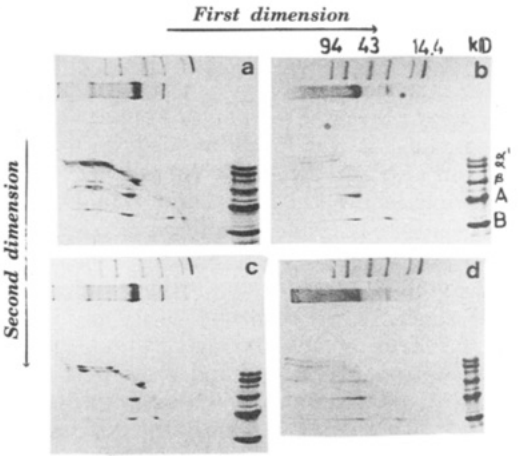

Figure 2. Bidimensional SDS–PAGE of soybean protein isolates: first dimension, without β-mercaptoethanol (ME); second dimension, with ME; (a, b) SPI 2; (c, d) SPI 1. Extraction solutions: (a, c) buffer pH 8–SDS–urea; (b, d) distilled water. Low molecular mass markers used are included in the first-dimensional SDS–PAGE: phosphorylase *b* (94 kDa); albumin (67 kDa); ovalbumin (43 kDa); carbonic anhydrase (30 kDa); trypsin inhibitor (20.1 kDa); α-lactalbumin (14.4 kDa).

charge at pH 8.0, and the electrostatic repulsion can explain the high solubility of SPI 2 in DW. An effect of salting-out was reported at an ionic strength of 0.1 (Hermansson, 1978), which agrees with the lower solubility of SPI 2 in B (ionic strength of about 0.1). On the other hand, the presence of urea and SDS would produce a solubilization by disruption of noncovalent bindings, explaining the high solubility of SPI 2 in BSU.

The protein solubility of SPI 1 in DW was lower than in B and BSU, probably because the pH is near the p*I* and protein–protein interactions are expected to be increased.

The electrophoretic patterns of BSU extracts from SPI 1 and 2 were very similar (Figure 2a,c). Samples that were not treated with ME show high molecular mass aggregates (>94 000 Da, some of them did not enter the gel), the α, α′ and β subunits of β-conglycinin, the AB subunit, and the A polypeptide of glycinin. The patterns also show a faint band in the usual region for the B polypeptide of glycinin and the soybean trypsin inhibitor (Figure 2a,c; first dimension, without ME). These electrophoretic patterns were described by Sathe et al. (1987). A and B polypeptides were observed after treatment with ME, which was expected because these

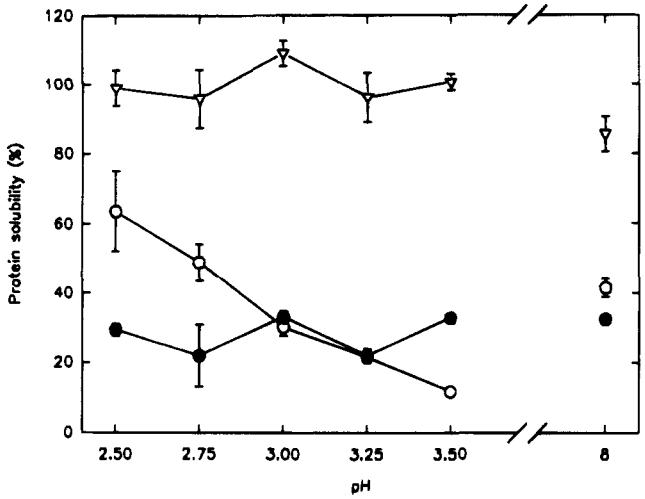

Figure 3. Protein solubility of heat-induced (90 °C, 30 min) gels (10% protein, w/w) from SPI 1 and SPI 2 vs pH of the gel: SPI 1, pH 2.50–3.50; SPI 2, pH 8. Protein concentration for all solubilization assays was 0.2% w/w. Extraction solutions: (○) distilled water, (●) buffer pH 8; (▽) buffer pH 8–SDS–urea. The bars show standard deviation.

subunits were associated by disulfide bonds (Figure 2a,c; first dimension) as was found by Moreira et al. (1979) and Nielsen (1985). Two-dimensional SDS–PAGE of BSU extracts show that the high molecular mass aggregates consisted mainly of α and α′ subnits of β-conglycinin and small amounts of A and B polypeptides of glycinin (Figure 2a,c; second dimension).

No differences were observed between the electrophoretic patterns of DW extracts from SPI 1 and 2 (Figure 2b,d) or between DW and B extracts from both SPI preparations (data not shown). Samples that were not treated with ME show mainly the AB subunit and an aggregated B polypeptide of glycinin. Small amounts of high molecular mass aggregates which did not enter the gel, formed by α, α′, and β subunits of β-conglycinin, and other proteins which entered the gel that consisted mainly of α and α′ subunits of β-conglycinin are also observed (Figure 2b,d; second dimension).

Protein Solubility of SPI Gels. The minimum protein concentration required for the formation of gels from SPI 1 was 7% w/w. A protein concentration of 10% w/w was utilized to study the effect of pH, and protein concentrations between 8 and 14% w/w were used to analyze the effect of protein concentration on gel characteristics.

Figure 3 shows the protein solubility of gels of SPI 1 in the function of pH. The solubility of the protein constituents in DW decreased with increasing pH in the pH range 2.5–3.5. This result was expected because the positive charge of the protein was progressively neutralized when the pH approached the p*I*, favoring protein aggregation. Protein solubility of gels in B (μ of about 0.1) was 20–30% (Figure 3). The low solubility in this buffer had been observed in the case of SPI powders and was attributed to an effect of salting-out at this ionic strength. The low solubility of gel proteins in B indicates that electrostatic forces would not be involved in maintaining the gel. Nakamura et al. (1986), Utsumi and Kinsella (1985a,b), and Yamauchi et al. (1991) investigated gels performed at pH 7.6–8.0 and concluded that hydrogen and disulfide bonds are involved in the maintaining of gel structure. The high protein solubility of gels in BSU (about 100%, Figure 3) indicates that gels are not stabilized by disulfide

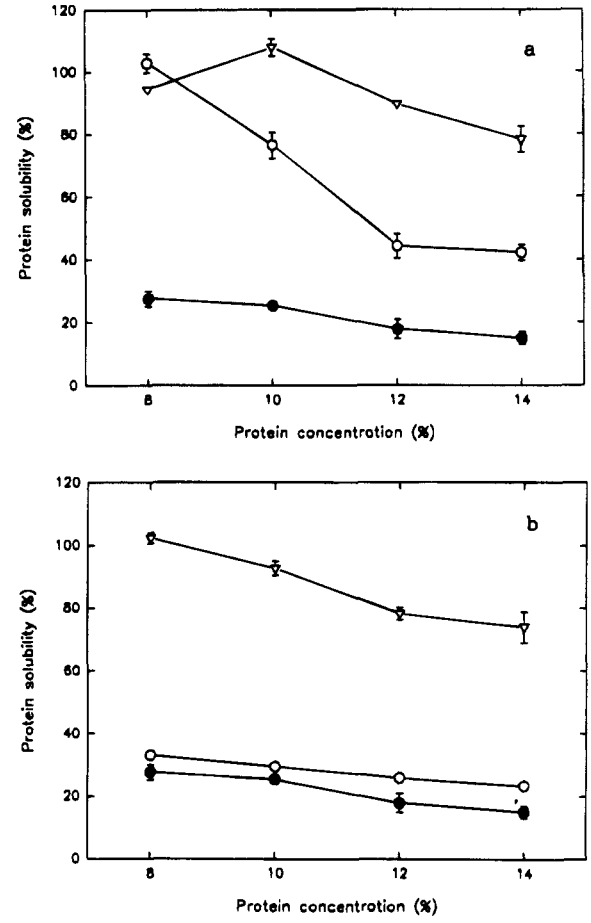

Figure 4. Protein solubility of heat-induced (90 °C, 30 min) gels from SPI 1 vs protein concentration: (a) pH 2.75; (b) pH 3.50. Protein concentration for all solubilization assays was 0.2% w/w. Extraction solutions: (○) distilled water; (●) buffer pH 8; (▽) buffer pH 8–SDS–urea.

bonds. This fact was expected because sulfhydryl–disulfide interchange reactions do not occur, at least to a high extent, at acidic pH, and thus, gels can be completely solubilized by urea and SDS, which cause the disruption of noncovalent bindings. Similar results were observed in acid milk whey protein gels (Lupano et al., 1992) in which protein was almost completely solubilized by BSU.

Protein solubility in B and BSU was independent of the pH of the gel, which indicates that in these cases the extraction conditions were more important in the maintenance or disruption of gel structure than the conditions of gel formation.

A gel at pH 8.0 was prepared under the same conditions. Protein solubility of this gel in DW was intermediate between that in gels of pH 2.75 and 3.0, and protein solubility in pH 8.0 buffer was similar to that of acid gels. On the other hand, protein solubility in BSU was only about 85%, probably due to the presence of disulfide bonds. Similar values were obtained in gels at pH 8.0 by other researchers (Shimada and Cheftel, 1988).

Results indicate that both SPI gels prepared at pH 2.75 and 3.0 are stabilized by noncovalent bonds, whereas at alkaline pH disulfide bonds are also involved in the maintaining of the gel structure.

Figure 4 show the effect of protein concentration at pH 2.75 and 3.50 on gel characteristics. Protein solubility in DW, B, and BSU decreased with the gel protein concentration. Solubility in DW decreased with increas-

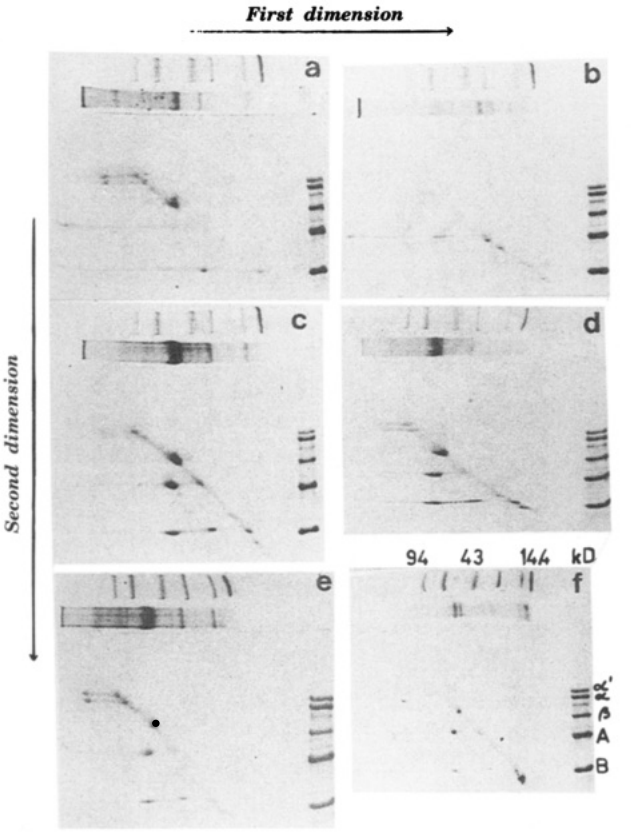

Figure 5. Bidimensional SDS–PAGE of heat-induced (90 °C, 30 mim) gels (10% protein, w/w) from SPI 1 and SPI 2: first dimension, without ME; second dimension, with ME; (a, b) SPI 2; (c, d, e, f) SPI 1; (a, c, e) buffer pH 8–SDS–urea; (b, d, f) distilled water; (a, b) pH 8; (c, d) pH 2.75; (e, f) pH 3.50.

ing protein concentration more sharply at pH 2.75 than at pH 3.50; at pH 3.50 protein solubility was very low even at low gel protein concentration. At a more acid pH (far from the p*I*) protein–protein interactions would be enhanced only by a high protein concentration (Figure 4a), whereas at a pH close to the p*I*, protein aggregation would be favored also by a decrease in the net charge of the protein (Figure 4b). The high protein solubility of gels in BSU, even at high gel protein concentration, indicates that gels would be stabilized essentially by noncovalent bonds. Protein solubility in BSU was less than 100% in gels performed at a protein concentration of 14%. This could be attributed to an insufficient dispersion of the gel in the extraction solution or to the presence of disulfide bonds, which would be favored by the high protein concentration.

Electrophoresis of Gel Extracts. The BSU extracts from gels of SPI 1 and 2 showed different one-dimensional electrophoretic patterns (Figure 5a,c,e). Bands of molecular mass higher than 94 000 Da (one of them did not enter the gel), bands corresponding to the α, α′, and β subunits of β-conglycinin, and others corresponding to free and aggregated B polypeptide were observed in BSU extracts from SPI 2 gels (pH 8.0), whereas the band of the AB subunit is diminished (Figure 5a, first dimension). The high molecular mass band that entered the gel was formed by α and α′, as observed by the bidimensional electrophoresis (Figure 5). Utsumi et al. (1985b) reported the involvement of disulfide linkage in the aggregation of β-conglycinin during gelation. Aggregates of B polypeptides linked by disulfide bonds were also observed (Figure 5a; second dimension), which agrees with results found by Utsumi

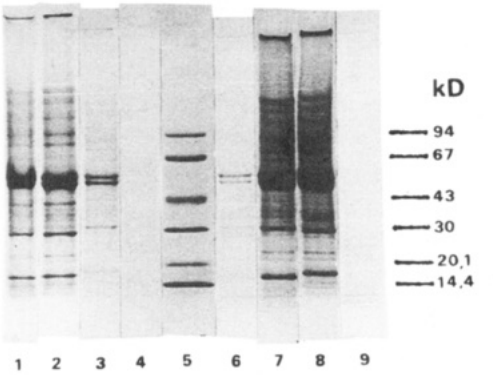

Figure 6. SDS–PAGE of heat-induced (90 °C, 30 min) gels from SPI 1: (1–4) pH 2.75; (6–9) pH 3.50. Gel protein concentration: (1, 2, 6, 7) 8% w/w; (3, 4, 8, 9) 14% w/w. Extraction solutions: (1, 4, 6, 9) distilled water; (2, 3, 7, 8) buffer pH 8–SDS–urea; (5) LMW.

et al. (1984) in heat-induced dispersions (0.25–0.5%, pH 8) of soy proteins.

The BSU extracts from SPI 1 gels also show all of these bands and another one corresponding to the AB subunit of glycinin (Figure 5c,e).

Distilled water extracts from acidic gels (pH 2.75 and 3.50) showed mainly the AB subunit of glycinin and the β subunit of β-conglycinin and small amounts of α, and α′ subunits of β-conglycinin and A and B polypeptides of glycinin. High molecular mass aggregates, formed by α and α′ subunits, free and aggregated B polypeptides, and low molecular mass peptides (<20 000 Da), were also observed (Figure 5d,f). More of the low molecular mass peptides were observed in the pH 3.50 gel.

On the other hand, the DW extract from SPI 2 gel presented a large amount of polypeptide A, in the monomer form or polymerized peptides linked by disulfide bonds. Similar results were reported by Yamauchi et al. (1991). This extract also showed small amounts of α, α′, and β subunits of β-conglycinin (Figure 5b). No bands were observed in the SDS–PAGE of B extracts from SPI 1 and SPI 2 gels.

Electrophoretic patterns of BSU and DW extracts were very similar at both acidic pH and protein concentrations (Figure 6). Band intensities agree with the results of protein solubility described in the preceding section (Figure 4).

The AB subunit was extracted with BSU in both SPI 1 and SPI 2 and from acidic gels, but not from the gel at pH 8.0. Similar behavior was observed in DW extracts: in acidic gels there were mainly AB subunits, β subunits, and B aggregates, which did not appear in the gel at pH 8.0. It is known that sulfhydryl–disulfide interchange reactions and the formation of β–B aggregates are favored at pH 8.0; thus, the glycinin fraction (AB subunit and B polypeptide) and the β subunit of β-conglycinin would contribute to the pH 8.0 gel structure, whereas β-conglycinin would be more important in the maintenance of acid gel networks.

Water-Holding Capacity (WHC) and Scanning Electron Microscopy (SEM) of Gels. Acid gels showed a high WHC, which decreased with increasing pH in the pH range 2.50–3.50. The WHC of the pH 8.0 gel was close to those of more acid gels (Figure 7). Similar results were observed by Shimada and Cheftel (1988) in gels of milk whey protein isolate. These proteins have a p*I* in the same range as soy proteins. At a pH near the p*I* protein–protein interactions increase and protein–water interaction decrease as a consequence of electrostatic repulsion. The gels at pH

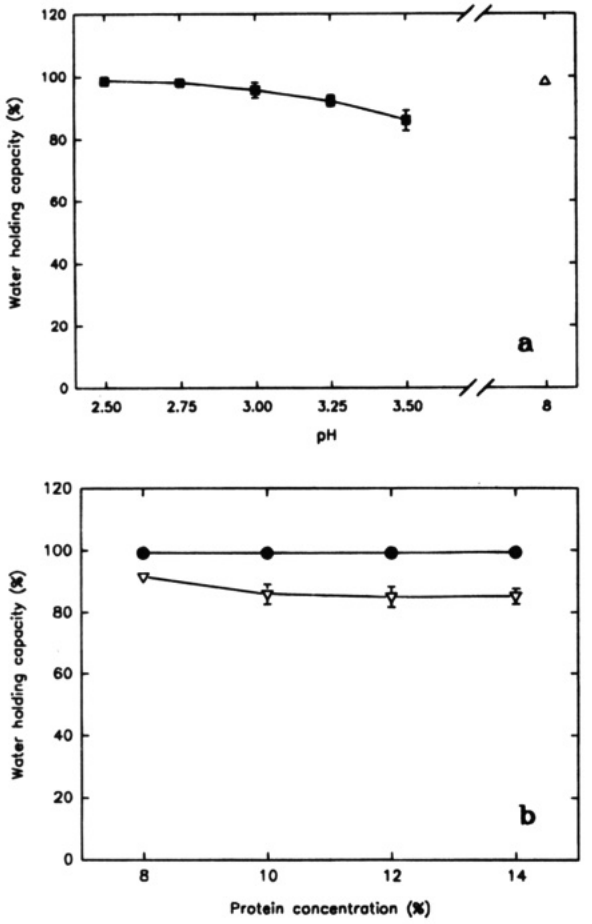

Figure 7. Water-holding capacity (WHC) of heat-induced (90 °C, 30 min) gels from SPI 1 and SPI 2: (a) vs pH (10% protein, w/w) (■) SPI 1, (△) SPI 2; (b) vs gel protein concentration (●) SPI 1, pH 2.75, (▽) SPI 1, pH 3.50.

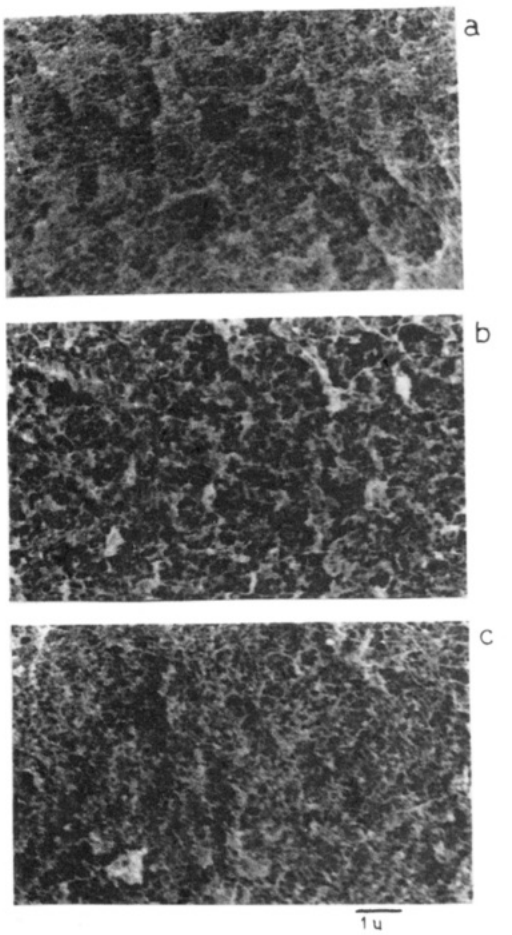

Figure 8. Scanning electron microscopy (SEM) of heat-induced (90 °C, 30 min) gels (10% protein, w/w) at pH (a) 2.75, (b) 3.50, and (c) 8.0. Magnification: 12000×. (This figure is reproduced here at 50% of the original size.)

3.5 have a microstructure more aggregated and less homogeneous than those at pH 2.75 and 8. Gels at pH 8.0 present a network similar to that of more acidic gels but with a different molecular distribution (Figure 8). Moreover, we observed that gels at pH 3.5 are less transparent than those at pH 2.75 and 8.

Figure 7 shows the WHC of gels as a function of protein concentration, at pH 2.75 and 3.50. The WHC remained practically constant with increasing protein concentration in the gel at pH 2.75, whereas a small decrease was observed in the gel at pH 3.50. In this case protein–protein interactions would be favored by a decrease in the net charge of the protein and also by a high protein concentration.

Conclusions. Results show that both acid and pH 8 soy protein isolates present high solubility and similar protein composition.

On the other hand, extracts of heat-induced gels obtained from these isolates show electrophoretic patterns different from the corresponding isolates, indicating that there are certain protein species which are more involved in the maintenance of the gel structure. Solubility in distilled water decreases with protein concentration of gels, but solubility in buffer pH 8 and buffer pH 8–SDS–urea is not affected by protein concentration. Acid gels are different from pH 8 gels, as confirmed by SDS–PAGE and SEM analysis. Acid gels, also, became more aggregated when pH aproached p*I*.

These facts would suggest different applications of these products as food ingredients.

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