Structural Characterization of Amaranth Protein Gels

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ABSTRACT: Gelation capacity of a native amaranth protein isolate was studied. Structural properties of gels prepared at different protein concentration and heating conditions were analyzed. Proteins present in amaranth isolates obtained by water extraction at pH 9.0 and subsequent isoelectric precipitation are able to form gels of yellowish appearance. Gel color intensity increased while luminosity decreased with increasing protein concentrations. High protein concentration allowed the formation of matrices with high water-holding capacity. In addition, increasing the heating temperature resulted in gels of high luminosity and low water-holding capacity. The increase of protein concentration (10% to 20% w/v) as well as the increase of heating temperature (70 °C to 95 °C) and heating time (10 to 30 min) resulted in the formation of a more ordered matrix with smaller pores, mainly stabilized by disulfide bonds and, at a lower extent, by noncovalent interactions (specially hydrogen bonds and hydrophobic interactions). Both amaranth globulin (11S globulin and P globulin) participated in gel structure via high-molecular-weight aggregates (>100 kD). Gel structure was stabilized via noncovalent bonds by monomer species of 42 kD and those of molecular mass lower than 20 kD localized in the interstitial spaces of gel matrix.

Keywords: amaranth proteins, gelation, gel microstructure, water imbibing capacity, gel color

Introduction

Amaranth is a cereal-like crop whose grains contain an important amount of proteins (17% w/w) with a well-balanced essential amino acid composition, rich in lysine and sulfur-containing amino acids (Teutónico and Knorr 1985; Martinez and Añón 1996; Castellani and others 1999). Amaranth proteins are able to complement cereal or legume proteins and can be used, due to their high nutritional value, as ingredients in food formulations (Martinez and Añón 1996). Because of its composition, properties, and current applications, amaranth is an underused crop with high food potential. Flour obtained from amaranth seeds is being increasingly used in tortillas, breads, cookies, pastas, and breakfast cereals. The use of amaranth isolates in food recipes depends largely on the functional properties of their component proteins (Marcone and Kakuda 1999), which in turn are directly related to their structural characteristics. Common functional properties required by a protein isolate include adequate solubility, water and fat absorption, heat coagulation, gelation, emulsifying, and foaming properties (Damodaran 1989; Marcone and Kakuda 1999).

Although much is known about physicochemical properties of amaranth protein isolates and their fractions (Bressani and García-Vela 1990; Martínez and Añón 1996; Martínez and others 1997; Castellani and others 1998, 1999; Marcone and others 1998a, 1998b; Goristein and others 2001), little information is available about functional properties of these isolates (Shinghal and Kulkarni 1991). Konishi and Yoshimoto (1989) observed an increase in emulsifying activity of salt-soluble amaranth globulin with thermal treatment, due to the increase in protein surface hydrophobicity. Marcone and Kakuda (1999) observed high foaming capacities and emulsifying activities over a wide pH range (3 to 9) for an amaranth globulin isolate.

A major challenge is to incorporate amaranth into existing food formulations to modify their functional and nutritional quality, as well as to create entirely new products such as gel-like products. Gelation properties of several proteins, including those from soybean (Damodaran 1988; Kang and others 1991; Puppo and others 1995; Puppo and Añón 1998a, 1998b, 1999; Renkema and others 2001; Molina and others 2002; Molina and Ledward 2003), whey (Walkenström and others 1998; Boye and others 2000; Lowe and others 2003), ovalbumin (Van Kleef 1986; Doi and others 1987; Rossi and Schiraldi 1992), and protein mixtures (Comfort and Howell 2002; Olsson and others 2002), have been widely studied, but no information about gelation properties of amaranth proteins is available.

The protein network formed during the gelation process can be considered the result of a balance of protein-protein and protein-water interactions and of attraction and repulsion forces occurring between adjacent polypeptide chains (Matsumura and Mori 1996). Attraction forces include noncovalent (hydrophobic, electrostatic, and hydrogen bonds) and covalent interactions (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 the pH and ionic strength of the medium (Utsumi and Kinsella 1985a, 1985b; Damodaran 1988; Puppo and others 1995). Repulsion forces, which act mainly at pH values far from the pI, and protein-water interactions help to keep polypeptide chains separated, favoring the formation of a homogeneous matrix (Heertje 1993).

Disulfide bond formation on heating produces an irreversible gelation process on ovalbumin (Van Kleef 1986) and β-lactoglobulin (Dumay 1988; Clark and others 2001) gels. By contrast, gelatin gels are reversibly stabilized, mainly by hydrogen bonds (Ledward 1986).

Gelation capacity of amaranth proteins could improve the production of lysine-enriched bakery products such as creams and puddings. Amaranth protein gels could also replace dairy products in lactose-intolerant patients.

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The objective of the present work was to study the gelation capacity of a native amaranth protein isolate and the structural properties of gels prepared at different isolate concentration, heating times, and thermal conditions.

Materials and Methods

Amaranth protein isolate

Amaranth protein isolate was prepared from defatted amaranth meal (Martínez and Añón 1996). Seeds were harvested at Estación Experimental del Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), Chapingo, Mexico. Flour was obtained by grinding the whole seeds in a Udy mill (Facultad de Ciencias Agrarias y Forestales-UNLP, Argentina), 1-mm mesh, and screened by 10-xx mesh. Lipids were extracted during 24 h with hexane (10% w/v) at 4 °C under continuous stirring. Flour was air-dried at room temperature and stored at 4 °C until used. Amaranth flour was dispersed in distilled water (1:10, w/v). The dispersion was adjusted to pH 9.0 with 2 *N* NaOH, stirred at room temperature for 30 min, and centrifuged at 9000 × *g* for 20 min at 15 °C. The supernatant was adjusted to pH 5 with 2 *N* HCl and centrifuged at 9000 × *g* for 20 min at 4 °C. The pellet was suspended in distilled water, adjusted to pH 7 with 0.1 *N* NaOH, and freeze-dried. Protein content of flour and isolate determined by the Kjeldhal method were 24.7% ± 1.3% and 85.5% ± 1.1% (w/w) (Nx5.85) (Segura-Nieto and others 1994; Martinez and Añón 1996), respectively. Because of the pH used for protein extraction, this isolate was named API9.

Gelation of amaranth protein isolate

Aqueous dispersions of amaranth protein isolate (API9) of different isolate concentrations (10%, 15%, and 20%, w/v) were placed in glass tubes (1.5-cm inner dia × 6-cm height) with tightly closed stoppers. Gelation was performed by heating the glass tubes in a water bath at different temperatures (70 °C, 80 °C, 90 °C, and 95 °C) and for different times (10, 20, and 30 min). The thermal-treated tubes were cooled immediately in a water bath at 15 °C. Gel samples were kept at 4 °C for 24 to 48 h before analysis.

The effect of isolate concentration was analyzed on gels prepared heating API9 dispersions at 90 °C and 95 °C during 20 min. Temperature effect was studied on 15% w/v gels heated 20 min, while the effect of heating time was analyzed on 15% w/v gels heated at 95 °C.

Color properties of gels

Color properties of gels were measured in Chroma Meter CR-300C colorimeter (MINOLTA, Osaka, Japan). Several parameters of gels were measured: luminosity (L^*), equilibrium between green and red (a), and equilibrium between yellow and blue (b). Surface color attributes such as color (hue, H*) and intensity of color (chroma, C*) were calculated from those parameters, as follows:

$$H^* = 1/\tan(b/a) \qquad a > 0 \qquad (1)$$

$$H^* = 180 + 1/\tan(b/a) \qquad a < 0 \text{ y } b \geq 0$$

$$C^* = (a^2 + b^2)^{1/2} \qquad (2)$$

Parameters were measured in different gel zones. An average of 10 measurements was carried out for each gel.

Scanning electron microscopy

Selected gel portions were 1st immersed in 2.5% glutaraldehyde containing 0.1% w/v ruthenium red 0.025 *M* KCl for 72 h. Samples were washed several times with 0.025 *M* KCl followed by 2% w/v OsO_4 treatment (2 h at 4 °C). Afterward, gels were rinsed 1 h in distilled water before being dehydrated in a graded 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 (SEM), at an acceleration voltage of 5 kV.

Solubility of gels

Gel samples (0.5 g) were dispersed in different extraction media (2.5 mL): distilled water (W), 0.1 *M* buffer phosphate-pH 7 (P) and in the same buffer containing 0.5% SDS (PS), 8 *M* urea (PU), or 0.5% SDS plus 8 *M* urea (PSU). Gel dispersions were stirred during 30 min and then centrifuged 15 min at 10000 × *g* at 15 °C. Protein solubility of gels was determined according to the Lowry method (Lowry and others 1951).

Electrophoresis

One-dimensional and 2-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on the gel extracts used for protein determination, according to the Laemmli method (Laemmli 1970). A continuous 12% separating gel and a 4% stacking gel were used. A continuous dissociating buffer system was used, which contained 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. Protein solutions were diluted with an equal volume of sample buffer (0.125 *M* Tris-HCl, 0.1% SDS, 40% v/v glycerol, 0.05% bromophenol blue, pH 6.8). Each 1st-dimension slab gel portion was treated with 10 volumes of SDS buffer (62.5 m*M* Tris-HCI pH 6.8–1% SDS–0.2 *M* β-mercaptoethanol [ME]–20% sucrose) for 30 min at 55 °C with 2 changes of solution. Treated gels were placed on the top of the second-dimension SDS-slab gel. The electrophoresis was carried out at a constant voltage of 200 V. Gels were fixed and stained with 0.1% R-250 Coomasie Brilliant Blue in water/methanol/acetic acid solution (5:5:20) for 12 h and were de-stained with water/methanol/acetic acid (65:25:10). Low MW markers (Pharmacia calibration kit) used included phosphorylase b (94 kD), albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), trypsin inhibitor (20.1 kD), α-lactalbumin (14.4 kD).

Water-holding capacity of gels

Each gel (0.3 to 1.3 g) was equilibrated at room temperature and placed on a nylon plain membrane (5.0-mm pores, Micronsep, New York, N.Y., U.S.A.) 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 (Puppo and others 1995). Water-holding capacity (WHC) was expressed as the percentage of the initial water remaining in the gel after centrifugation. Each value is the mean (± standard deviation) of at least 4 determinations.

Statistical analysis

A 2-way model of analysis of variance (ANOVA) was used to statistically analyze data (Statistix 1.0, Tallahassee, Fla., U.S.A.; 1996 Analytical Software Window 95). The significance of differences among means of the several treatments was determined by the least significance difference (LSD) test at $P \leq 0.05$.

Results and Discussion

Thermal conditions (temperature and heating time) and protein concentration of dispersions strongly influence the thermal gelation of proteins (Clark and Lee-Tuffnell 1986). Gel formation by

globular proteins is a complex process, which often involves several reactions such as denaturation, dissociation-association, and aggregation (Renkema 2001). In the denaturation process, a native protein unfolds and functional groups become exposed and can interact with each other to form aggregates. When protein concentration is high enough, aggregation leads to the formation of a gel. There is a minimum protein concentration, a critical concentration (Cc) for gel formation (Foegeding 1989). The type of gel that is formed—fine-stranded or coarse-aggregated networks—depends on conditions during gel formation (pH, protein concentration, and heating temperature). In general, gels become coarser as the pH approaches the isoelectric point or when the ionic strength is increased (Renkema 2001).

In previous studies, we have shown that proteins from amaranth isolates present a thermogram with 2 endotherms (69.9 °C and 98.8 °C) (Martínez and Añón 1996; Avanza and others 2003) and that they can gellify at protein concentrations higher than 7%. In addition, we have found that these gels present viscoelastic characteristics and that their rheological properties depend on treatment time and temperature and on protein concentration (Avanza and others, 2005). In the present study, we characterized amaranth gel structure by SEM and analyzed color and water retention capacity of protein matrices formed under different treatment conditions. Solubility assays of the matrix in different extraction media and analysis of the polypeptides extracted (SDS-PAGE) allowed us to predict the forces involved in the stabilization of the network and the nature of proteins that participate of gel structure.

Color properties of gels

Color parameters of amaranth protein gels are shown in Table 1 as a function of treatment conditions. The predominant color, related to H^*, was close to yellow in all cases. H^* values decreased significantly ($P < 0.05$) at isolate concentration higher than 10% w/v. Besides color intensity of gels (C^*) increased and luminosity (L^*) decreased with increasing protein concentrations ($P < 0.05$). Variation in color properties would be attributed to increased protein-protein interactions. Color properties of gels (L^* and C^*) also diminished with the duration of the thermal treatment used for gelation ($P < 0.05$). In contrast, no variation in color (H^*) of gels with heating time was observed. Gel color varied differently with treatment temperature; the yellow color (H^*) diminished at temperatures higher than 80 °C, without changes in intensity (C^*) but with a marked increase in luminosity (L^*) ($P < 0.05$). With the increase in heating temperature, a major proportion of protein molecules are denatured. This leads to an increase in the number of hydrophobic and/or hydrophilic groups exposed to the environment, thus favoring the interaction between proteins. The different behavior of C^* and L^* parameters with temperature and protein concentration showed that protein-protein interactions favored by heating differed from those favored by a high protein content.

Color properties of gels are of great importance due to the addition of this kind of products to different formulated foods. Application of different protein isolates in gel-like products would be limited by inadequate color properties.

Table 1—Effect of isolate concentration (95 °C, 20 min), heating temperature (15%, 20 min), and heating time (95 °C, 15%) on color properties of amaranth protein gels[a]

Color parameters	Isolate concentration (%)		
	10	15	20
H^*	89° 70′ ± 1.34[a]	87° 10′ ± 0.42[b]	85° 53′ ± 1.06[b]
L^*	74.94 ± 0.81[a]	73.31 ± 0.26[b]	71.67 ± 0.93[c]
C^*	7.86 ± 0.42[a]	8.50 ± 0.16[b]	10.27 ± 0.53[c]
	Heating temperature (°C)		
	70	80	95
H^*	94° 53′ ± 1.64[a]	82° 20′ ± 0.48[b]	87° 01′ ± 0.42[c]
L^*	58.02 ± 0.61[a]	64.82 ± 0.52[b]	73.59 ± 0.26[c]
C^*	8.02 ± 0.27[a]	10.82 ± 0.90[b]	8.50 ± 0.16[a]
	Heating time (min)		
	10	20	30
H^*	87° 32′ ± 0.31[a]	87° 01′ ± 0.42[a]	86° 10′ ± 0.77[a]
L^*	74.14 ± 0.24[a]	73.59 ± 0.26[b]	72.66 ± 0.32[c]
C^*	9.44 ± 0.06[a]	8.50 ± 0.16[b]	7.72 ± 0.08[c]

[a]Different letters in the same line are significantly different ($P < 0.05$).

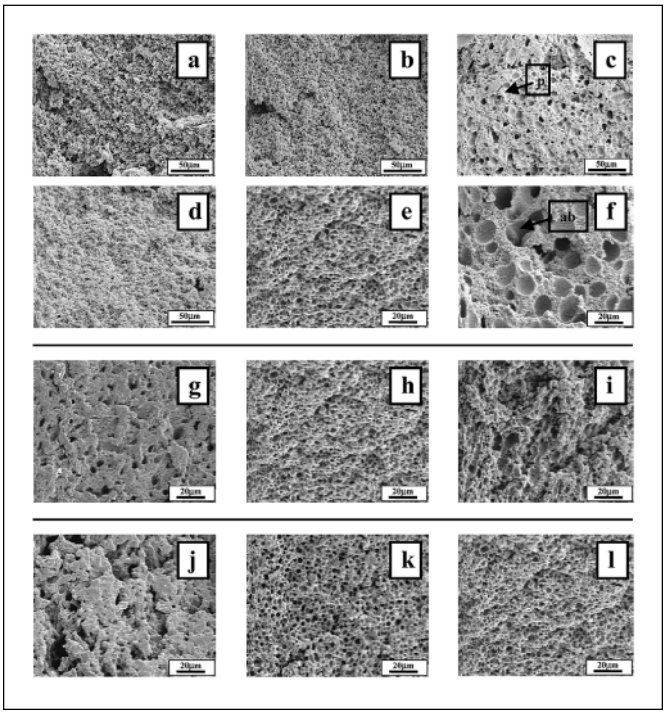

Figure 1—Effect of isolate concentration (a through f) (a, d: 10%; b, e: 15%; c, f: 20%): (a, b, c) 90 °C, 20 min, (d, e, f) 95 °C, 20 min; heating time (g through i) (g: 10 min; h: 15 min; i: 20 min) (15% w/v, 95 °C) and heating temperature (j through l) (j: 70 °C; k: 80 °C; l: 95 °C) (15% w/v, 20 min) on microstructure of amaranth gels determined by SEM. p = protein matrix; ab = air bubble.

Scanning electron microscopy

Micrographs of gels are shown in Figure 1. For gels heated at 90 °C (Figure 1a through 1c) and 95 °C (Figure 1d through 1f) for 20 min, the increase in isolate concentration produced a reduction of pore size. This suggests the formation of a more compact matrix as a consequence of the increase in protein-protein interactions. When isolate concentration and temperature were kept constant, an increase in heating time resulted in an increase matrix order (Figure 1g through 1i). Treatment times longer than 10 min were required to obtain an ordered protein network with small pores. A similar tendency was observed when treatment temperature was increased while keeping constant protein concentration (15% w/v) and heating time (20 min). A disordered matrix was formed at 70 °C, but its structure increased with increasing gelation temperatures (80 °C and 95 °C) (Figure 1j through 1l). Treatment at temperatures close to or higher than the denaturation temperature, 69.9 °C and 98.8 °C, for amaranth isolate proteins resulted in the unfolding of

molecules, and the interaction between chains led to the formation of a gel with a more structured matrix.

An increase in protein concentration results in an increase in protein-protein interactions due to the higher number of molecules in the sample. While the total number of polypeptides is conserved, the temperature increase allows a higher number of molecules to exceed the dematuration temperature (Td); because of the increase in unfolded molecules, interactions also increase. In both cases, the increased interaction yields a more structured matrix, which results in color diminution (H^*). At concentration (15%) and temperature (95 °C) over critical gelation values, the increase in the heating time results in a longer exposure of the molecules to the treatment temperature, thus favoring the formation of a more structured matrix without color change (H^*) but with low color intensity (C^*).

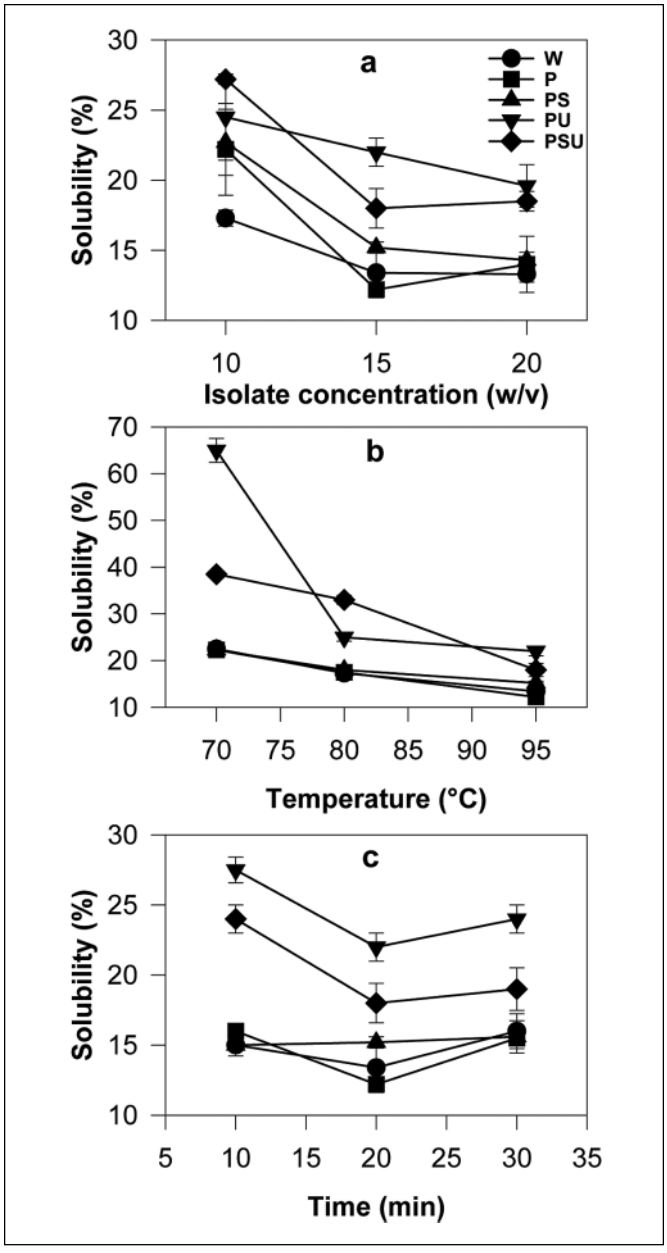

Figure 2—Effect of isolate concentration (a), heating temperature (b), and heating time (c) on protein solubility (%) of amaranth gels dispersed in different extraction media: ■ = distilled water (W); ● = phosphate buffer (P); ▲ = phosphate buffer + SDS (PS); ▼ = phosphate buffer + urea (PU); and ◆ = phosphate buffer + SDS + urea (PSU).

Protein solubility of gels

To predict the nature of the interactions that stabilized the matrix of amaranth gels, the solubility of gels in different media was analyzed. Results are shown in Figure 2. Solubility in all the tested media, containing chaotropic agents but no reducing agents, was less than 30%, suggesting that the gel matrix is stabilized mainly by covalent bonds of the disulfide type (Figure 2a). The increase in protein concentration also reduced the solubility in all the tested media (Figure 2a). Solubility assays in the presence of β-mercaptoethanol were also performed and in all cases gels dissolved completely. In addition, it must be kept in mind that amaranth proteins, mainly P globulin and 11S globulin, have a high SH/SS content.

Gels at 10% w/v concentration showed low water (W) solubility, which increased with the addition of denaturing agents (SDS an urea) (PS, PU, PSU) ($P < 0.05$). For gels at 20% w/v, in contrast, a slight increase in solubility was observed only with the addition of urea, irrespective of the presence of SDS ($P < 0.05$). These results suggest that noncovalent intermolecular forces have a higher resistance to rupture as a consequence of a greater protein-protein interaction favored by a closer proximity among chains and/or by the formation of disulfide bonds. This phenomenon was observed in previous studies with soy protein gels (Puppo and others 1995).

Figure 2b shows the variation of gel solubility with increasing gelation temperature. Solubility in W, P, and PS was almost unmodified and stayed at values lower than 20%. This fact would indicate that the forces that keep protein molecules linked within the gel matrix are not of electrostatic nature. In the presence of urea and at 70 °C (PS70), a great increase in solubility was observed (65%), which further decreased with the addition of SDS (38%) (PSU). Both denaturing agents (urea and SDS) affect protein conformation by altering noncovalent bonds (Gorinstein and others 1996). Urea interferes mainly with hydrophobic interactions inside the protein and with hydrogen bonds in the polar regions, whereas SDS interacts mainly with hydrophobic links and, given its negative charge, could also interact with positively charged protein groups. The observed phenomenon would indicate that gels of weak structure stabilized primarily by noncovalent hydrophobic interactions are formed at 70 °C. At higher temperatures (80 °C and 90 °C), close to denaturation temperatures, a matrix stabilized mainly by disulfide bonds would be formed, as can be deduced from the very low solubility values (<25%) in media containing urea and SDS ($P < 0.05$).

The effect of heating time is shown in Figure 2c. Solubility in W, P, or PS did not change with heating time and remained between 12% and 16%. The addition of urea (PU) or urea and SDS (PSU) to the buffer resulted in an increased solubility, specially for gels obtained at short heating times (10 min). The mild destabilization produced by these denaturing agents would indicate that a matrix stabilized 30% by noncovalent links is formed, whereas disulfide bonds would play a more important role at longer heating times.

Analysis of proteins present in soluble fractions

Extracts in different media of gels obtained at different temperatures (20 min, 15% protein) yielded similar SDS-PAGE patterns. High-molecular-weight aggregates not entering the gel, soluble aggregates of 143 and 121 kD, polypeptides of 65, 56, 52, 42, 35, 28, and 20 kD, and low-molecular-weight peptides (<20 kD) were observed.

To analyze the nature of soluble aggregates and proteins present in each extract, bidimensional electrophoresis was per-

formed under denaturing and dissociating conditions (Figure 3). Water extracts from gels obtained at 70 °C (W70) yielded an electrophoretic pattern characterized by the presence of high-molecular-weight soluble aggregates (143 and 121 kD) formed by polypeptides of 56, 32, and 22 kD, dimers of 32 and 22 kD polypeptides (A and B 11S polypeptides, respectively), a high proportion of 56-kD polypeptides (P globulin), and peptides of 42, 14.4, and 20 kD. This pattern is in line with that obtained by Martínez and Añón (1996) for an amaranth protein isolate at pH 9. High-molecular-weight aggregates were not observed in the water-soluble fraction of gels obtained at 95 °C (W95). The denaturing temperature of amaranth proteins present in the API9 isolate has been surpassed at 95 °C, leading to the formation of an structured matrix, mainly stabilized by high-molecular-weight aggregates not soluble in water. Polypeptides of 56 and 42 kD and low-molecular-weight peptides (<20 kD) linked by polar noncovalent interactions remain in the interstitial spaces of the matrix. At 80 °C (W80), an intermediate situation was observed. Water was able to extract a low proportion of aggregates of high molecular mass in addition to the polypeptides extracted in W95.

To simplify visualization and interpretation, only patterns of phosphate buffer-soluble proteins in the presence of urea (PU) were shown in Figure 3. Phosphate buffer-soluble extracts (P) of gels, at all temperatures, presented an electrophoretic pattern identical to that yielded by the aqueous extract (data not shown). The addition of SDS to the phosphate buffer (PS) did not disrupt the gel matrix, as shown by the existence of the same protein species in the electrophoretic pattern of PS and P extracts (data not shown).

Extracts soluble in phosphate buffer containing urea (PU) of gels obtained at 70 °C (PU70) exhibited a larger proportion of 56 and mainly 42 and 33 kD (A-11S) polypeptides (Figure 3) than that observed in phosphate buffer extracts with and without SDS (PS70 and P70), which agree with the higher solubility in this buffer (Figure 2). At 80 °C, urea allowed a high proportion of 33 kD (A-11S) to be extracted compared with W80, P80, and PS80. No differences were observed between P95 and PU95 electrophoretic patterns. Urea and SDS jointly were unable to disrupt forces that maintain gel structure formed at temperatures higher than denaturation temperatures. The addition of urea and SDS to the phosphate buffer (PSU) resulted in the extraction of a higher proportion of high-molecular-weight soluble aggregates (>100 kD) from matrices of gels obtained at 70 °C and 80 °C, indicating the presence of hydrogen bonds and hydrophobic interactions that are disrupted by these chaotropic agents (data not shown). This phenomenon was not observed at 95 °C.

Results at 80 °C confirm that gel network is developed by aggregates formed by P-56 (56 kD) and 11S globulin (A-11S, 33 kD, and B-11S, 20 kD). These subunits would be stabilized by noncovalent (hydrophobic and hydrogen) and disulfide bonds. The fact that no increase in protein extractability in the presence of chaotropic agents for gels obtained at 95 °C was detected, would indicate that an increase in heating temperature led to the formation of a great number of disulfide bonds. These disulfide bonds would impede the total disruption of gel matrix. Otherwise, the partial extraction of P-56 and the 42 kD polypeptides indicate that these species were weakly linked to the gel matrix or occupying the gel interstices.

Water-holding capacity of gels

It is well known that the WHC is directly related to the type of matrix formed. Gels with a more ordered matrix present high WHC values, whereas gels that develop a coarse, disordered, and open network are not able to retain water and show low WHC (Hermans-

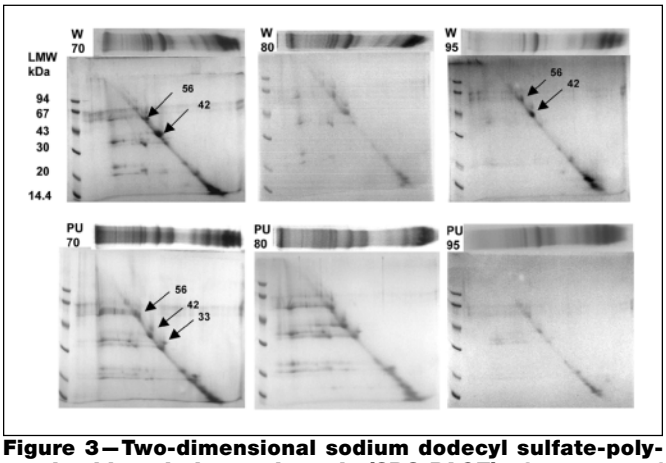

Figure 3—Two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of extracts of gels (15% w/v, 20 min of heating) prepared at several temperatures (70 °C, 80 °C, and 95 °C) and extracted with different extraction media: distilled water (W) and phosphate buffer + urea (PU).

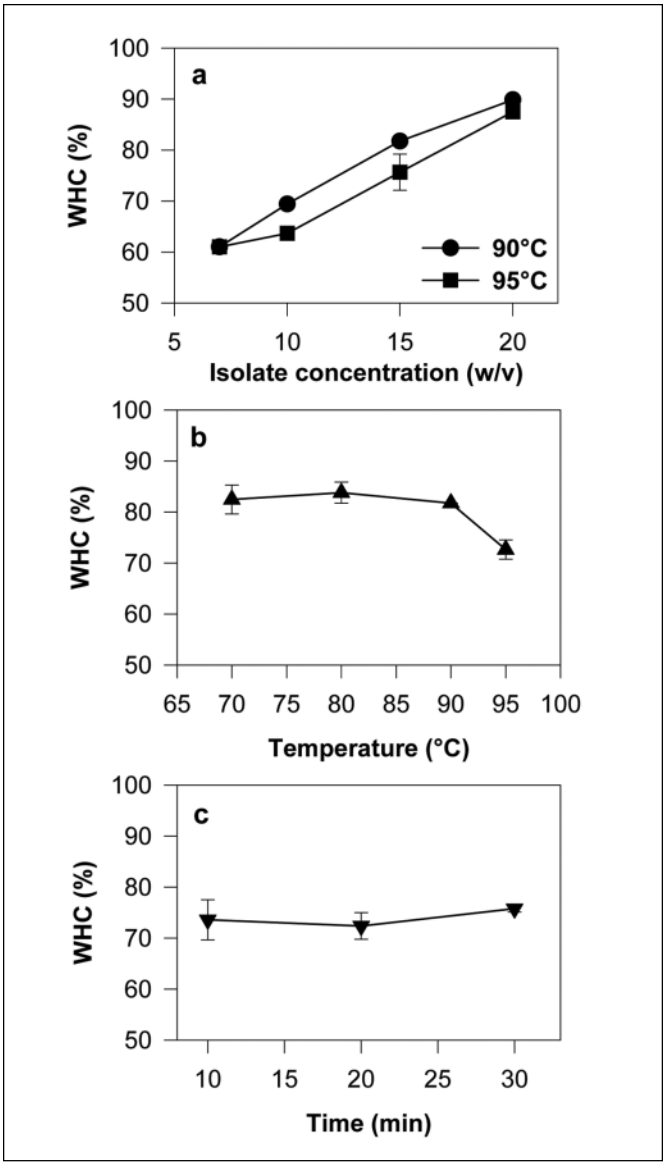

Figure 4—Effect of isolate concentration (a), heating temperature (b), and heating time (c) on water-holding capacity (WHC [%]) of amaranth gels.

son 1994; Puppo and others 1995; Puppo and Añón 1998b). Figure 4a shows the variation of WHC with protein concentration. We can observe that the higher the isolate concentration, and consequently the protein concentration, the higher the WHC of gels ($P < 0.05$). These results agree with those obtained by SEM (Figure 1). The formation of a more compact matrix as a result of a larger protein-protein interaction by disulfide bonds, led to a higher water retention. Increasing the heating temperature beyond 90 °C resulted in the decrease of the WHC of gels ($P < 0.05$), probably due to the total denaturation of amaranth proteins (Figure 4b).

An increase of heating time up to 30 min did not modify the WHC of gels ($P < 0.05$) (Figure 4c). Despite WHC results, protein molecules require a minimum time to orientate before interacting and forming gels with a more ordered matrix and higher structure. This phenomenon was more evident in SEM micrographies (Figure 1).

It is important to remark that gels obtained at 70 °C formed a more disordered and less stable matrix, although with a notable WHC, whose aggregates were easily extracted with distilled water and, in a higher proportion, with PSU. Gels obtained at 95 °C had a more structured matrix with low WHC because it was mainly stabilized by high-molecular-weight aggregates not extractable with any of the extraction media assayed. Such aggregates might be formed by monomers with molecular masses 56, 35, and 28 kD stabilized by noncovalent bonds such as hydrophobic interactions and hydrogen bonds.

Comparison of gel properties between amaranth and soybean proteins

Gelation properties of other vegetable proteins such as soybean proteins, which like amaranth proteins are mainly composed of 11S globulin, were deeply studied by several authors (Utsumi and Kinsella 1985a, 1985b; Puppo and others 1995; Renkma and others 2002; Renkema 2004). Soybean protein gels of neutral pH, just as amaranth gels, present a minimum protein concentration for gelation of approximately 7%. These gels are transparent and develop high values of WHC (>90%). Unlike amaranth, gels were opaque with a great aggregation degree and a lower WHC than soybean gels. In both cases, depending on thermal conditions used for gelation, gels with more aggregated structure were less transparent and showed lower WHC than gels with a more ordered network. Unlike amaranth proteins, the increase in protein concentration did not modify WHC of soybean gels. In a different way from amaranth proteins, matrices of soybean protein gels are stabilized mainly by noncovalent bonds. Due to sulfhydryl-disulfide interchange reactions favored at pH 8, soybean gels present a non-aggregated and ordered structure stabilized by AB subunit of glycinin and β-subunit of β-conglycinin. Otherwise, amaranth gels presented a structured matrix formed by high-molecular-weight aggregates stabilized mainly by disulfide bonds. Those aggregates were also stabilized by 56-, 42-, and 20-kD monomers linked by noncovalent bonds.

Conclusions

Proteins present in amaranth isolates obtained by water extraction at pH 9.0 and subsequent isoelectric precipitation are able to form gels of yellowish appearance. The increase of protein concentration (10% to 20%) as well as the increase of heating temperature (70 °C to 95 °C) and heating time (10 to 30 min) resulted in the formation of a more ordered matrix with smaller pores, mainly stabilized by disulfide bonds and, at a lower extent, by noncovalent interactions (specially hydrogen bonds and hydrophobic interactions). Gel matrix is mainly formed by high-molecular-weight aggregates composed by 11S globulin (35- and 28-kD polypeptides) and P-globulin (52-kD monomer) and by the nonaggregated 11S and P globulins. Interstitial spaces of gel matrix lodges the 42-kD and low-molecular-weight (14.4 and 20 kD) monomer species. These polypeptides stabilize gel structure via noncovalent interactions. At concentrations lower than 15% w/v, temperatures close to 70 °C, and heating times shorter than 15 min, a more disordered matrix was formed, with a predominance of covalent interactions but with an important proportion of proteins bounded by hydrogen bonds and hydrophobic interactions.

Due to the increased demand for new based protein foods to cope with the diverse nutritional needs and taste of people, amaranth proteins would be a suitable ingredient in gel food formulation.

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