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Rheological characterization of amaranth protein gels

M.V. Avanza[a], M.C. Puppo[b], M.C. Añón[b,*]

[a]Facultad de Ciencias Exactas, Universidad Nacional del Nordeste Av. Libertad 5400, 3400 Corrientes, Argentina
[b]Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA) (UNLP-CONICET), 47 y 116, 1900 La Plata, Argentina

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Abstract

Gel forming properties of amaranth proteins at different thermal conditions and protein concentration were studied. Gel point (G' and G'' crossover) and gelation kinetics (G' vs. time) were analyzed. The type of gel formed from the rheological point of view was studied analyzing the rheograms obtained from frequency sweeps. Texture properties of cold-set gels were analyzed by TPA assays. Minimum conditions for gelation were 7%, w/v and 70 °C. Elasticity of heated dispersions and gels increased with the increase of protein concentration. A high value of the network structure index was observed. This behavior could be related to the great proportion of disulfide bonds formed during amaranth protein gelation. At temperatures above 70 °C (80, 90 and 95 °C), gelation of dispersions (15%, w/v) took place at times less than 5 min. A first order kinetic gelation process with reaction rate specific constant values that increased with the increase of heating temperature was observed. A rapid denaturation of globulins followed by sulfhydryl/disulfide interchange reactions between protein molecules conduced to a gelation phenomenon enhanced by protein aggregation. Gels prepared over critical conditions ($T > 70$ °C, protein concentration $> 7\%$, w/v) presented a strong gel-like behavior. These type of gels were elastic in nature (tan $\delta < 0.1$), of high hardness, fracturability and cohesiveness, although presented low adhesiveness. Depending on protein and thermal conditions, amaranth proteins were able to form self-supporting gels that could be applied in different gel-like foods.
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Keywords: Amaranth proteins; Gelation; Gel texture; Gel viscoelasticity; Kinetic gelation

1. Introduction

Interest in amaranth grain has increased in recent years because of its high nutritional value as well as some agricultural advantages such as relative high grain yield, resistance to drought, and short production time (Mendoza & Bressani, 1987). Amaranth is a dicotyledoneous plant with a well balanced protein content, and has been proposed as a new alternative source of high quality protein (Castellani, Martínez, & Añón, 2000). Amaranth proteins contain acceptable levels of essential amino acids, particularly lysine, tryptophan, and methionine, which are found in low concentrations in cereals and leguminous grains of common usage (Mendoza & Bressani, 1987; Teutónico & Knorr, 1985). Structural characteristics of these proteins influence their functional properties. While the physicochemical properties of amaranth proteins have been widely studied (Abugoch, Martinez, & Añón, 2003; Bressani & García-Vela, 1990; Castellani, Martinez, & Añón, 1998, 1999; Goristein et al., 2001; Marcone, Kakuda, & Yada, 1998a,b; Martínez & Añón, 1996; Martinez, Castellani, & Añón, 1997), their functional properties were not deeply analyzed.

Albumins, which are involved in diverse biological functions, and globulins, which are storage proteins, are the major protein fractions of amaranth seed (Castellani et al., 1998). The main amaranth globulins are the 11S type globulin and the highly polymerized globulin defined as globulin-P by Castellani et al. (1998), both having similar structural and physicochemical properties to those of legumin 11S globulins (Castellani et al., 1999).

One of the most important functional properties of proteins is gelation. The phenomenon of heat-induced globular protein gelation was extensively described in several reviews (Clark, Kavanagh, & Ross-Murphy, 2001;

* Corresponding author. Tel.: +54 221 424 9287; fax: +54 221 425 4853.
E-mail address: mca@biol.unlp.edu.ar (M.C. Añón).

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Clark & Lee-Tuffnell, 1986; Grinberg, Grinberg, Bikbov, Bronich, & Mashkevich, 1992). Taking into account the increase in the nutritional value of amaranth grain with thermal processing (Mendoza & Bressani, 1987) and the lack of studies on the gelation properties of amaranth proteins, we decided to study gelation capacity of these proteins. A great 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 capacity and gel properties are straightforward related to their rheological properties (Ross-Murphy, 1995). Protein gels are composed by a protein matrix within which the aqueous phase is occluded. Rheological properties such as viscoelasticity and texture are closely related with microstructure of the matrix gel. The gels of fine-stranded matrix (Foegeding, Bouland, & Hardin, 1995), are harder and retain more water than those of more open matrices (particulate gels). The contributions of covalent and non-covalent bonds to gel texture and viscoelasticity are different in nature. Disulfide bonds usually play an important role in increasing gel matrix hardness whereas hydrogen and hydrophobic interactions used to be responsible for keeping network structure (Puppo & Añón, 1998; Zheng, Matsumura, & Mori, 1993).

The aim of the present work was to study the gelation kinetic of amaranth proteins and deepen the study of the influence of thermal treatment and protein concentration on viscoelastic and textural properties of amaranth protein gels.

2. Materials and methods

2.1. Amaranth protein isolate

Amaranth protein isolate was prepared from defatted amaranth meal (Martínez & Añón, 1996). Seeds were harvested at Estación Experimental del Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), Chapingo, México. 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 $9000\times g$ for 20 min at 15 °C. The supernatant was adjusted to pH 5 with 2 N HCl and centrifuged at $9000\times g$ for 20 min at 4 °C. The pellet was suspended with distilled water, adjusted to pH 7 with 0.1 N NaOH and freeze-dried. According to the extraction pH this isolate was named API9. Protein content of flour and isolate, as determined by the Kjeldahl method, were 24.7 ± 1.27 and 85.5 ± 1.14% (w/w) (wb) (N×5.85). API9 also contains 2.43 ± 0.25% of carbohydrates (w/w) (wb) and 10% of water. The yield obtained was 9.3 ± 1.0 g of API9 per 100 g of flour.

2.2. Viscoelasticity measurements

Linear viscoelasticity range was determined at 1 Hz frequency. Within this range, a 1% deformation was selected for frequency and time sweeps of all samples. Assays were performed in a Paar Physica MCR 300 (Stuttgart, Germany) rheometer, using a non-serrated parallel plate with a gap of 1 mm. For measurements during the gelation process, dispersions were placed at the thermostatized inferior plate. To prevent sample dehydration, plate edges were covered with low viscosity silicone. The US 200 V2.21 software was used for data analysis.

2.3. Gel point

Gelation capacity (G' and tan δ vs. time) of 15%, w/v aqueous dispersions of API9 was analyzed by measuring viscoelastic parameters at different heating temperatures (50, 60, 70, 80, 90 and 95 °C) during 20 min. Time for gelation (gel point) at each temperature was determined as the intersection between G' and G'' vs. time curves. Minimal conditions necessary to ensure gelation (critical temperature, T_{crit} and protein concentration, C_{crit}) were also determined. Values of temperature and protein concentration at which G' differed from G'', measured at 1 Hz frequency, were considered.

2.4. Gelation kinetics

It was studied by fitting experimental data with the following empirical formula (Puppo & Añón, 1998)

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

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).

2.5. Viscoelasticity characterization of amaranth dispersions

Dispersions of API9 isolate (15%, w/v) were thermally treated at different temperatures (50, 70, 80, 90 and 95 °C) during 20 min. Similar assays were performed with API9 dispersions of different protein concentrations (5, 7, 10 and 15%, w/v) that were heated during 20 min at 90 °C. After heating, all dispersions were cooled at 25 °C. Samples were characterized by rheological assays during heating (dispersions) and after cooling (gels). Measurements of G', G'' and tan δ vs. oscillating frequency were performed.

2.6. Gelation of amaranth protein isolate

Aqueous dispersions of amaranth protein isolate (API9) of different concentrations (10, 15, and 20%, w/v) were placed in glass tubes (1.5 cm i.d. ×6 cm height) with tightly closed stoppers. Gelation was performed according to Puppo, Lupano, and Añón (1995). Glass tubes were heated in a water bath at different temperatures (70, 80, 90 and 95 °C) and heating times (10, 20 and 30 min). After thermal treatment, tubes were cooled immediately in a water bath at 15 °C. Gel samples were kept at 4 °C for 24–48 h before texture profile analysis.

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

2.7. Texture profile analysis (TPA)

Gels (1.5 cm diameter and 1.5 cm of height) were compressed at 20 °C to 80% of their original height (1.5 cm) until rupture (Puppo & Añón, 1998) in a TA-XT2i Texture Analyser (Vienna Court, England). A plate–plate sensor system with a stainless probe SMSP/75 at a constant velocity of 0.5 mm/s was used. Texture of gels was analyzed by an uniaxial compression test of two cycles (TPA analysis) (Steffe, 1996). From each force vs. distance curve, parameters such as fracturability, hardness, adhesiveness and cohesiveness were obtained. In each determination, four gel samples were used and average values were calculated.

2.8. Statistical analysis

A two-way model of Analysis of Variance (ANOVA) was used to statistically analyze data (Statistix 1.0 USA; 1996 Analytical Software Window 95). The significance of differences among means of the several treatments was determined by the LSD test at $p \leq 0.05$.

3. Results and discussion

3.1. Gel forming properties

3.1.1. Determination of critical gelation concentration and temperature

Critical concentration (C_{crit}) and temperature (T_{crit}) conditions for gelation of an AP19 are shown in Fig. 1.

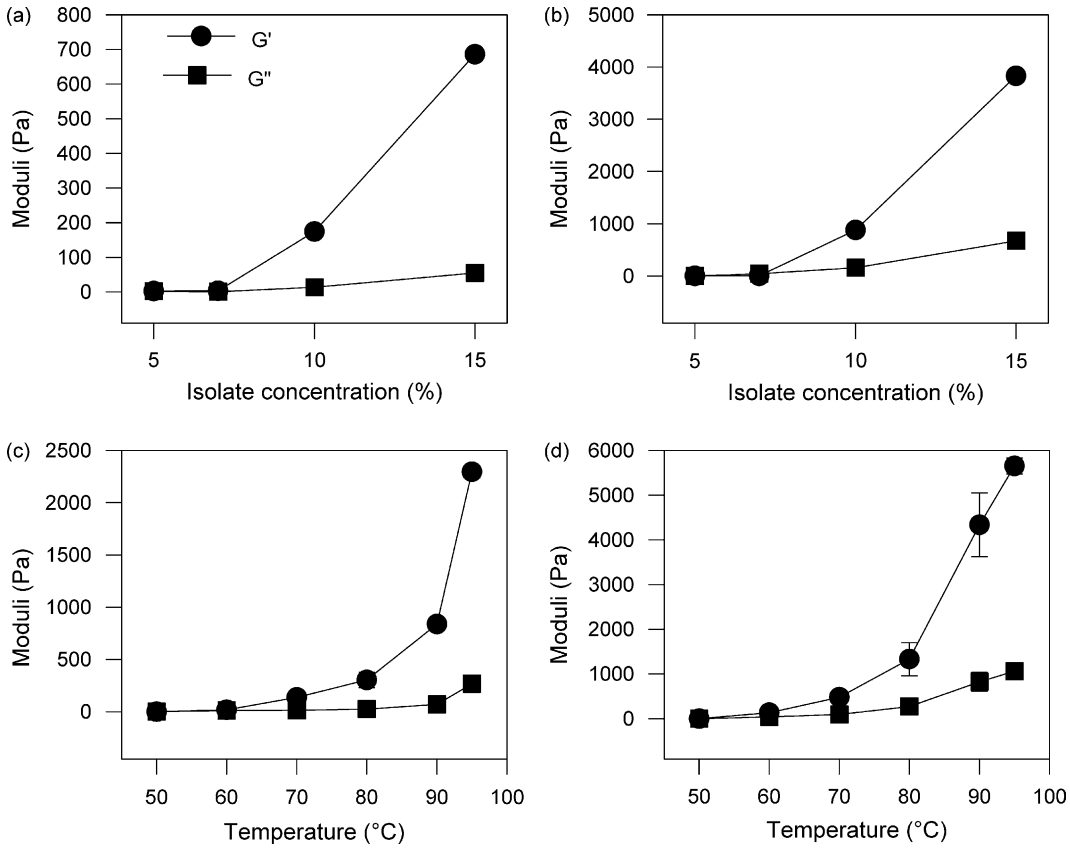

Fig. 1. Effect of amaranth isolate concentration (dispersions heated at 90 °C during 20 min) (a, b), and heating temperature (15% gels heated for 20 min) (c, d), on elastic (G') and viscous (G'') moduli of amaranth protein gels. Measurements performed: (a, c) after heating at 90 °C, (b, d) after heating at 90 °C and then cooling at 25 °C (gel setting).

The graphic depicts the change of the dynamic parameters G' and G'' (measured at a frequency of 1 Hz) with isolate concentration and temperature. C_{crit} and T_{crit} can be considered as the concentration and temperature at which G' begins to diverge from G''. Structures of low G' are formed under 7%, w/v, both after heating and after cooling (Fig. 1a and b). Increasing protein concentration over this value leads to an increase in both moduli, specially the elastic modulus, this effect being much more pronounced for gels (Fig. 1b). As a consequence, dispersions become more elastic ($G' > G''$) at concentrations higher than 7%, w/v.

The concentration dependence of the storage modulus, which gives information on the gelation efficiency and on the structure of the particle network (Renkema & van Vliet, 2004), can be approximated by a power-law function: $G \propto C^n$ (Clark, Ross-Murphy, Nishinari, & Watase, 1990). A power-law function between concentration and G' was obtained for API9 dispersions treated at 90 °C during 20 min, and after the setting process: $G' = aC^b$, where a is the origin coordinate and b is an index that reflects the nature of the association behavior and the network structure. For dispersions whose G' values were measured after heating, a and b values were 1.47×10^{-4} and 5.7 ($r^2 = 0.909$), respectively, while corresponding values for cooled gels were 1.94×10^{-5} and 7.1 ($r^2 = 0.859$). In most cases of biopolymers gels, the modulus is proportional to approximately C^2 at relatively high concentrations (10–20%, w/v) (Matsumura & Mori, 1996). An exponent value of 3.4 has been reported for heat-set soybean glycinin gel (2–7%) (Kohyama, Yoshida, & Nishinari, 1992). Renkema and van Vliet (2004) found an exponent value of 10.3 for a soybean protein isolate at pH 7. Similar exponent values indicate similar association behavior and similar network structure, although different chemical forces would be predominant in each type of gel. Previous studies performed in our laboratory on physicochemical characteristics of amaranth protein gels (Avanza, Puppo, & Añón, submitted for publication) showed that the increase in protein concentration leads to the formation of an ordered gel matrix mainly stabilized by disulfide bonds and, to a lower extent, by non-covalent interactions, specially hydrogen bonds and hydrophobic interactions. Therefore the higher values of b for amaranth protein gels, specially in cold-set type, could be due to the higher proportion of disulfide bonds present in the API9 gel matrix.

Values of G' and G'' obtained after heat processing of AP19 dispersions at different temperatures are shown in Fig. 1c and d. No differences were observed between the elastic and the viscous moduli at temperatures below 70 °C. This value was an inflection point above which, specially at 90 and 95 °C, G' was higher than G'' (Fig. 1c). Fig. 1d shows values of dynamic moduli (G', G'') obtained at 25 °C after cooling from the temperature achieved after heat processing at different temperatures. The same rheological behavior observed for dispersions after heating was founded after cooling process (Fig. 1d), although values for both moduli were higher. This behavior could be ascribed to the formation of hydrogen bonds during the cooling period, which may also be involved in the stabilization of the protein matrix.

Therefore, in agreement with Grinberg et al. (1992), minimal (threshold) concentration and temperature are needed to ensure an adequate gelation. In the case of amaranth proteins, such values would be 7%, w/v and 70 °C, respectively.

3.1.2. Gel point determination

To ensure the gelation process, samples must be heated at temperatures higher than the denaturing temperature of the different protein fractions, and at a concentration higher than the critical protein concentration. Previous studies (Martínez & Añón, 1996; Avanza & Añón, submitted for publication) have shown that the API9 has two denaturation endotherms, at 69.9 and 98.8 °C. The first endotherm corresponds to albumin denaturation, and the second one to the concomitant denaturation of 11S and P globulins (Martínez & Añón, 1996). Both denaturation and subsequent interaction of the different fractions are determining factors for the gelation kinetics of proteins present in amaranth isolates.

For a dispersion of a given concentration that is heated at a given temperature, the gel point could be defined (Pilosof, 2000) as the time at which G' and G'' intersect ($G' = G''$). This is an approximated method but the value obtained is usually close to the gel point. For our samples (15%, w/v) heated at 70 °C, the gel point was close to 5 min, and this value decreased by 25–30% at temperatures equal to or higher than 80 °C (Table 1). Therefore, the intersection took place at short heating times, specially when the main amaranth protein fractions were at least partially denatured ($T \geq 80$ °C). No intersection was detected at temperatures lower than T_d (50 and 60 °C) since G' and G'' values were very low and kept constant during the whole experiment (data not shown).

3.1.3. Gelation kinetics

Fig. 2 shows G' and tan δ variation as a function of heating time for 15%, w/v dispersions. As shown in the figure, the elastic modulus G' increased (Fig. 2a) and tan δ decreased (Fig. 2b) with temperature and heating time. The loss and storage moduli were not affected by heating temperatures higher than 90 °C. An elastic matrix

Table 1
Gelation time (G' and G'' intersection)

T (°C)	Gelation time (min)	$G' = G''$ (Pa)
70	5.33 ± 0.23	2.08 ± 0.19
80	3.99 ± 0.19	1.03 ± 0.20
90	3.58 ± 0.00	0.75 ± 0.01
95	3.67 ± 0.24	0.53 ± 0.13

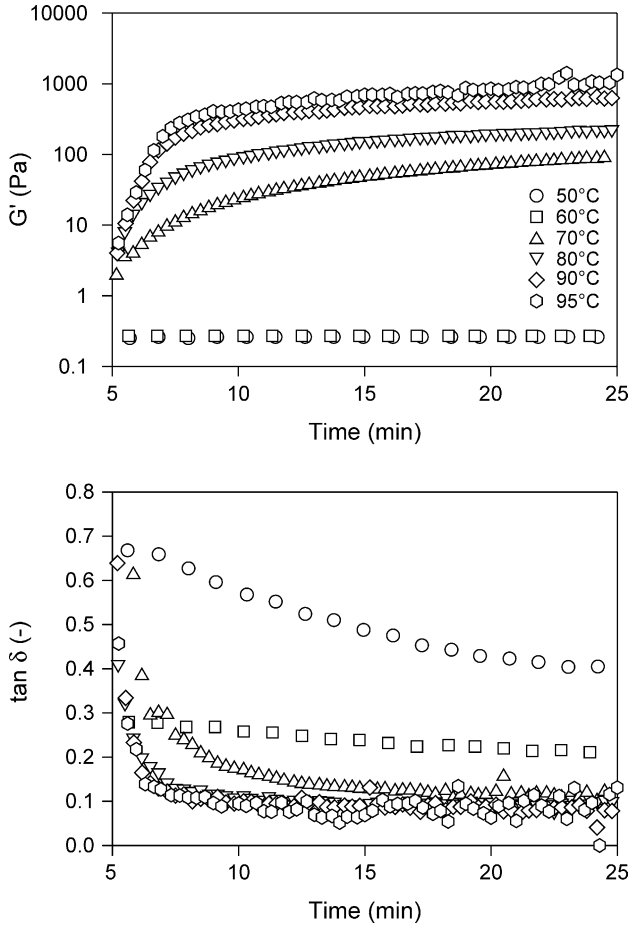

Fig. 2. Influence of thermal treatment on gelation kinetics (modulus vs. time) of amaranth protein dispersions (15%, w/v). (a) G', (b) tan δ (G''/G').

(tan $\delta < 0.1$) was formed at temperatures equal to or higher than 80 °C. The viscous component of the dispersion was higher at temperatures lower than the T_d (50 and 60 °C), yielding tan δ values higher than 0.25.

The kinetic parameters (k and G'_{sat}) calculated from G' vs. time curves (Fig. 2) are shown in Table 2. An increase in the reaction rate specific constant with gelation temperature was detected. Such increase was steeper at temperatures higher than 70 °C, with no significant differences between k values at 80, 90 and 95 °C. This behavior would indicate that gelation kinetics are mainly governed by the denaturation of species of lower thermal stability.

Table 2
Specific kinetic gelation constant (k) and G'_{sat}

Temperature (°C)	k (min^{-1})[a]	G'_{sat} (Pa)[a]
70	0.0186±0.0003	98.15±14.12
80	0.0402±0.0095	299.06±53.20
90	0.0429±0.0075	846.14±17.10
95	0.0444±0.0041	901.24±131.21

[a] $r^2 > 0.984$.

The saturation elastic modulus, G'_{sat}, increased steadily with temperature, showing almost a 10 fold increase for a 15 °C variation. Amaranth protein isolate dispersions at 15%, w/v concentration gelated at a constant rate over 70 °C, but the values of the elastic modulus depended on the heating temperature up to 90 °C.

Gelation kinetic constants of amaranth gels were similar to those of gels from soy protein isolates at pH 8 heated at 90 °C for 30 min and cooled at 4 °C for 48 h ($k \cong 0.043$ min^{-1}), while G'_{sat} values of soy proteins gels were lower (≅640 Pa) (Puppo & Añón, 1998). The higher G'_{sat} values of amaranth gels suggest a more structured matrix due to the formation, during the heating phase, of a higher proportion of disulfide bonds between globulins (11S and P globulins) and other protein fractions of the isolate (Avanza et al., submitted for publication). In the case of soy proteins, disulfide bonds are not the main crosslinks in soybean protein gels; non-covalent bonds play an essential role in the formation and stabilization of these matrices (Puppo et al., 1995; Van Kleef, 1986).

Changes in the elastic modulus directly reflect structural changes of the protein during the gelation process. Evidence based on IR and Raman spectroscopic analysis of various globular protein gels also suggests that β-sheet structure might be essential for protein–protein interactions and network formation in these gels. The intermolecular hydrogen bonds between β-sheets, oriented in either parallel or antiparallel β-sheet configurations, may act as junction zones and thus stabilize the gel network (Wang & Damodaran, 1991). Previous studies using FT-Raman measurements (Bosch et al., 2003) showed that AP19 presents a well-defined band between 1300 and 1200 cm^{-1}, with an important shoulder at 1234 cm^{-1} corresponding to a β-sheet conformation, typical of globulins. The exposure of aromatic hydrophobic amino acids increases during thermal denaturation, creating an environment that leads to the formation of aggregates due to intermolecular hydrogen bond and disulfide bond interactions. The AP19 aggregates formed by thermal treatment show a predominant β-sheet structure. The formation of this kind of secondary structure had a direct influence on the increase in storage modulus with heating temperature. The formation of a β-sheet structure, observed by FTIR, in both soybean β-conglycinin and glycinin subunits was also accompanied by an increase in the storage modulus. These results support the idea that heat-induced gels are formed by cross-linked with the intermolecular β-sheet structure in globular proteins (Nagano, Akasaka, & Nishinari 1994).

3.2. Rheological characterization of gels

3.2.1. Viscoelastic behavior

The nature of the gel formed under different thermal treatment conditions can be empirically determined by analysis of the frequency sweep (G' and G'' vs. frequency).

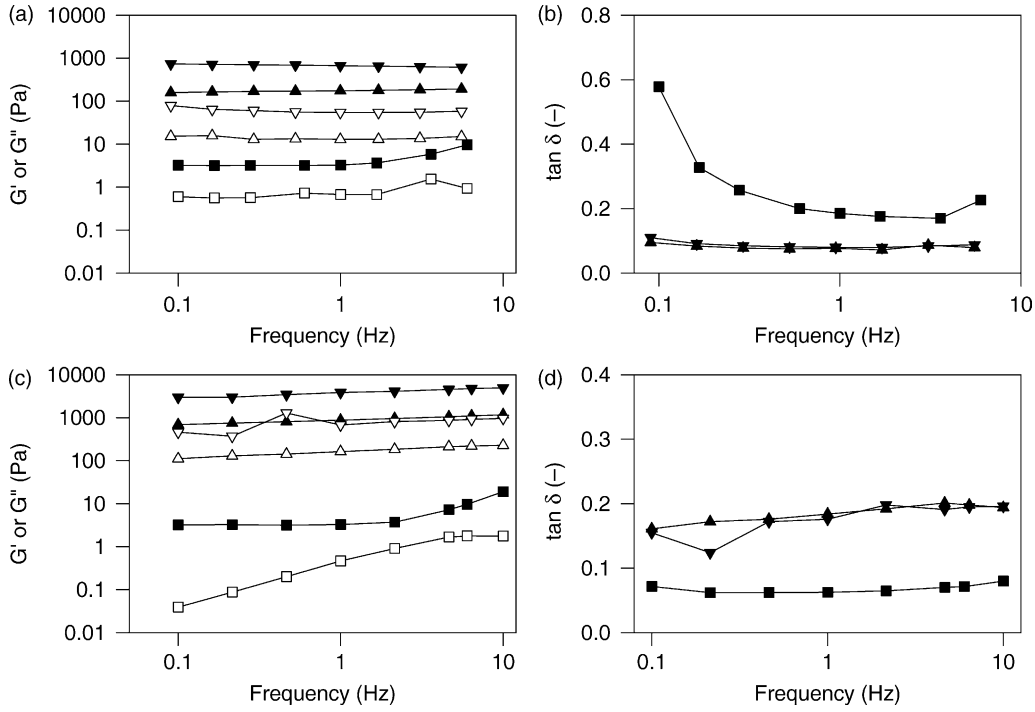

Fig. 3. Frequency sweeps (G' and G'' vs. f, tan δ vs. f) of amaranth protein dispersions prepared at different isolate concentrations. Thermal treatment: 90 °C—20 min. Measurements performed: (a, b) during heating, (c, d) after cooling (gel setting). Isolate concentration: (■, □) 7%, (▲, △) 10%, (▼, ▽) 15%. (■, ▲, ▼) G' and tan δ, (□, △, ▽) G''.

The influence of the isolate concentration on the viscoelastic properties of gels is shown in Fig. 3. The 5%, w/v dispersion presented a gradual G' increase with frequency after heating (data not shown), indicating a behavior typical of intercrossed solution (Ross-Murphy, 1995). At a 7%, w/v concentration, G' was higher ($f<1$ Hz), with values similar to those observed for the 5%, w/v dispersion at $f>1$ Hz (Fig. 3a). At higher concentrations (10 and 15%, w/v), G' was constant in the whole frequency range, with values close to 150 and 700 Pa, respectively. Gels at 10 and 15%, w/v exhibit a 'strong gel-like' behavior (Ross-Murphy, 1995), and their stability would be related to the formation of disulfide bonds because of a closer proximity of protein molecules.

As shown in Fig. 3b, the elasticity increased with protein concentration during thermal treatment, with tan δ values <0.1 for 10 and 15%, w/v dispersions. Cooling produced an increase of the elastic component of the less concentrated samples (5%, w/v), which presented a gel behavior typical of an intercrossed macromolecular solution (data not shown). The G' curves for gels at 7%, w/v presented very low values for the modulus ($\cong 3$ Pa) but with G' greater than G'' indicating a 'weak gel-like' behavior (Fig. 3c). The gels of higher concentration (10 and 15%, w/v) presented a marked G' and G'' increase as compared with heated dispersions with rheograms typical of strong gels. The elasticity of the 7%, w/v gels increased with cooling, while gels of higher concentration (10 and 15%, w/v) presented tan δ higher than those observed during heating (Fig. 3d). The small elasticity differences detected between the heated dispersions of higher concentration (panel b) and their respective gels (panel d) could be related to the fact that, after setting, hydrogen bonds may favor a minimal loss of gel elasticity.

The effect of thermal treatment on the viscoelastic characteristics of gels is shown in Fig. 4. The 15%, w/v API9 dispersion presented an intercrossed solution-like behavior at 50 °C, and a strong gel-like ehavior at 70, 80 and 95 °C (Ross-Murphy, 1995). The highest G' and G'' values corresponded to the dispersion heated at the highest temperature (Fig. 4a). A weak-gel behavior was observed at 60 °C, with tan δ higher than 0.6 throughout the whole frequency range (data not shown). At temperatures higher than 70 °C, the predominant rheological behavior was of the elastic type, with tan δ lower than 0.15 (Fig. 4b). The highest elasticity was exhibited by dispersions heated at 95 °C (tan $\delta<0.1$). This tan δ decrease could be due to a higher prevalence of hydrophobic interactions during the heating process. The elasticity of gels would be influenced mainly by the denaturation of 11S and P globulins, whose T_d are close to the highest heating temperatures (90 and 95 °C).

Cooling resulted in an increased G' and G'' moduli, with rheograms similar to those obtained during heating (Fig. 4c). However, tan δ exhibited a slight increase, reaching values close to 0.2 (Fig. 4d), which would indicate the formation of less elastic gels in which hydrogen bonds contribute minimally to matrix elasticity.

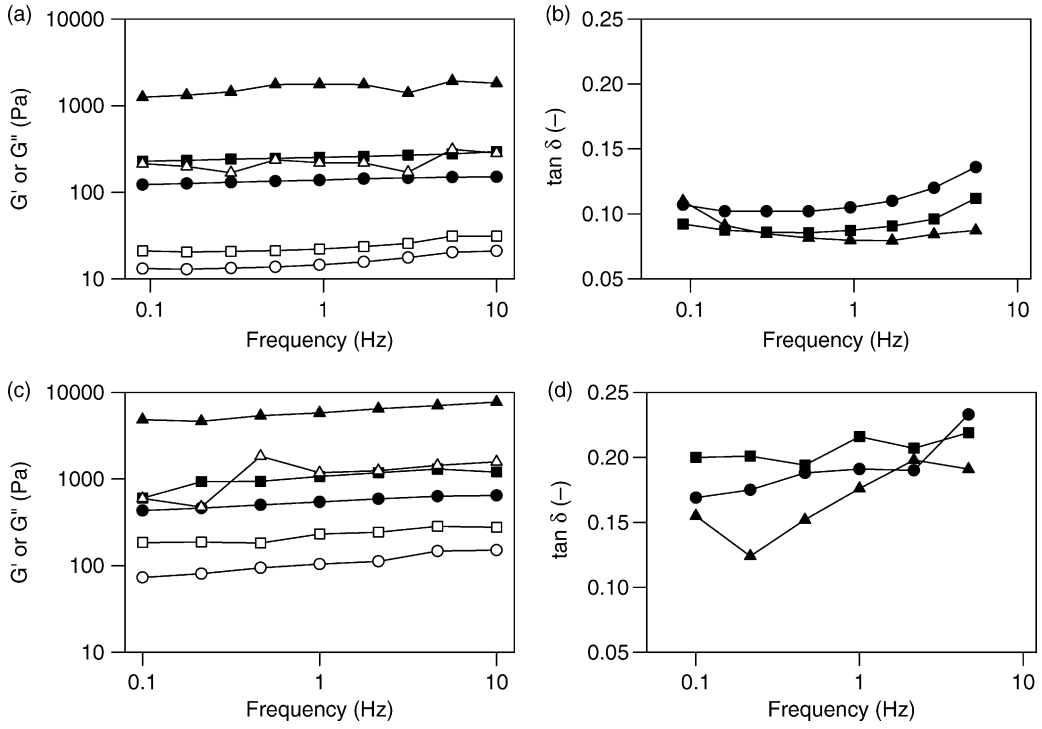

Fig. 4. Frequency sweeps (G' and G'' vs. f, tan δ vs. f) of amaranth protein dispersions prepared heating at different temperatures. Treatment conditions: 15%, w/v—20 min. Measurements performed: (a, b) after heating, (c, d) after cooling (gel setting). Thermal treatment: (●, ○) 70 °C, (■, □) 80 °C, (▲, △) 95 °C. (●, ■, ▲) G' and tan δ, (○, □, △) G''.

3.2.2. Texture profile

Fig. 5 shows the values for the texture profile parameters of gels prepared under different conditions. It can be seen that hardness and fracturability of gels increase with isolate concentration (10–20%, w/v). Gels are more cohesive and less adhesive as protein concentration increases.

The fracturability and hardness of gels augmented with temperature increase (70–95 °C), but the effect was lower than that produced by the increase in protein concentration. This could be explained by the fact that the globulin fraction, which is in higher proportion in the API9 isolate, is denatured at 95 °C, thus favoring the formation of disulfide bonds that are very important for the stabilization and hardness of gels. The adhesiveness of gels decreased significantly with heating temperature ($p < 0.05$), but their cohesiveness did not show significant changes. The hardness and fracturability of gels increased with heating time, while their adhesiveness decreased. In contrast, cohesiveness did not vary significantly with treatment time ($p > 0.05$) (Fig. 5).

An increase in protein–protein interactions results in an increased formation of disulfide bonds that would contribute to the hardness of gels from amaranth proteins. In a previous study (Avanza et al., submitted for publication) we found that, at high temperature and protein concentration, amaranth proteins form ordered gel matrixes stabilized mainly by disulfide bonds and, to a lower extent, by non-covalent interactions (specially hydrogen bonds and hydrophobic interactions). Gel matrix is mainly formed by high molecular weight aggregates composed of 11S globulin (35 and 28 kDa polypeptides) and P-globulin (52 kDa monomer), and by the non-aggregated 11S and P globulins. The 42 kDa and low molecular weight (14.4 and 20 kDa) monomeric species occupy the interstitial spaces of the gel matrix. These polypeptides stabilize the gel structure via non-covalent 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 linked by hydrogen bonds and hydrophobic interactions. The exposure of aromatic hydrophobic groups as a result of the heating process produces aggregates via hydrogen and disulfide interactions, generating a predominantly β-sheet type structure.

In other proteins, such as the 11S globulin from soy, highly concentrated samples contain a high proportion of soluble monomers and oligomers and of polymerized species stabilized by disulfide bonds. Thermal treatment leads to an increase of the molecular mass of soluble aggregates and a subsequent linear association between adjacent strands via hydrophobic interactions that lead to the formation of gel matrix (Yamauchi, Yamagishi, & Iwabuchi, 1991).

Stress at fracture and the elastic component were also higher in soybean protein gels with high content of covalent crosslinks (Comfort & Howell, 2002; Van Kleef, 1986). Similar results were obtained by Alting et al. (2004) using globular proteins (ovalbumin and whey proteins) that were

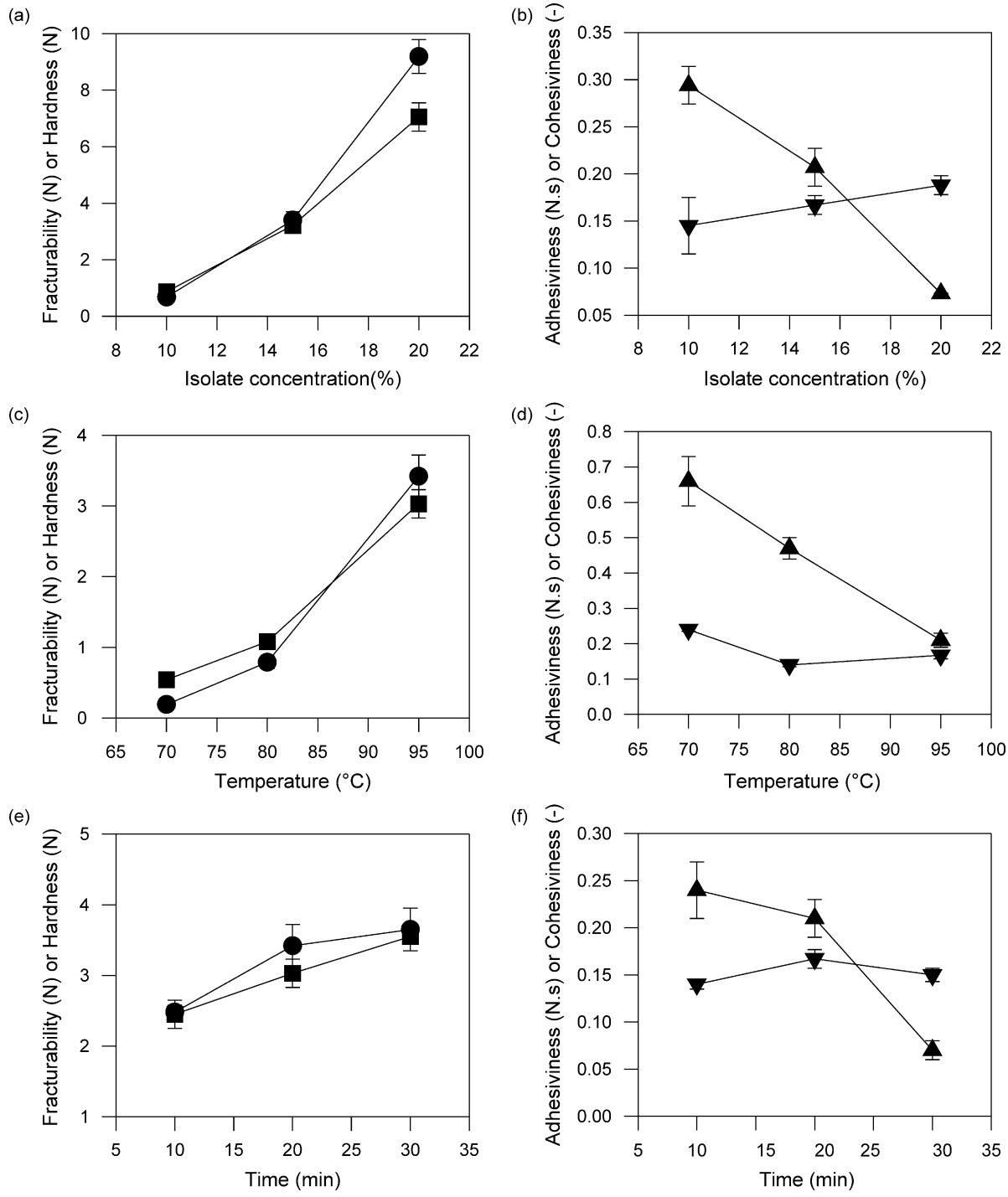

Fig. 5. Texture properties (TPA) of amaranth protein gels prepared at different isolate concentrations, heating temperature and heating time. (a, c, e) Fracturability (●) and hardness (■), (b, d, f) adhesiveness (▲) and cohesiveness (▼).

subjected to cold gelation induced by acid treatment. The formation of disulfide bonds was shown to be of great importance for the mechanical properties of gels and for their physical stability. A protein network was initially formed by physical interactions, which was subsequently stabilized by the formation of disulfide bonds (Alting et al., 2004). Disulfide bond formation in globular proteins of the 11S type seems to be influenced by changes in the secondary and tertiary structure during thermal treatment. The effect of protein concentration and thermal treatment on the structural changes of the gel matrix has been studied in other proteins. Using IR and Raman spectroscopy, Wang and Damodaran (1991) found that thermal treatment of 11S soy globulin increases the disordered structure (increased

random coil) at the expense of the β-sheet structure, yielding a matrix stabilized by electrostatic interactions, whose hardness increased with increasing protein concentrations. On the other hand, Ker, Chen, and Wu (1993), using FTIR measurement in solid phase, found that heating of the 11S soy globulin (10%) gives rise to a more ordered structure as a consequence of an increased α-helix content and a decreased random coil content. According to Ker et al. (1993), gels obtained at 80 °C present an aggregated structure of low hardness, while those obtained at 90 and 95 °C (T_d = 89.6 °C) present a very hard integral extended matrix, specially at 95 °C.

4. Conclusions

Amaranth proteins were able to formed self-supporting gels. Critical values of protein concentration and heating temperature of 7%, w/v and 70 °C were obtained, respectively. Elasticity of heated dispersions and gels increased with the increase in protein concentration. The index that reflects the entanglement of gel matrix, *b*, was high (7.1) and similar to that obtained for soybean protein gels and could be attributed to the high amount of disulfide bonds formed during gelation. At high temperatures ($T >$ 70 °C), elastic modulus increased with heating temperature leading a G' and G'' crossover (gel point) at low times of heating (<5 min). In consequence a rapid denaturation of 11S and P globulins was produced leading to gelation throughout disulfide interactions. A first order kinetic gelation behavior was observed. The gelation velocity was governed by the denaturation of fractions of the lowest thermal stability. The reaction rate specific constant increased with heating temperature, specially above 70 °C, without major changes between 80 and 95 °C. Gels obtained at high protein concentration and heating temperatures (above critical conditions) presented a viscoelastic behavior mainly elastic ($\tan\delta < 0.1$) similar to that of strong-like gels. These gels developed high hardness, fracturability and cohesiveness, although low values of adhesiveness were observed, mainly in gels prepared at the highest protein concentration (20%, w/v). We can conclude that it is possible to form self-supporting gels from amaranth proteins of different rheological properties depending on the requirements of applicability of these proteins in foods.

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