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Effect of acid treatment on structural and foaming properties of soy amaranth protein mixtures

J.L. Ventureira, E.N. Martínez, M.C. Añón*

Centro de Investigación y Desarrollo en Criotecnología de Alimentos (CIDCA), CONICET-CCT La Plata – Facultad de Ciencias Exactas, UNLP, calle 47 y 116, La Plata 1900, Pcia. de Buenos Aires, Argentina

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ABSTRACT

To obtain a food ingredient composed of soybean and amaranth proteins with better functionality, the proteins were subjected to an acid treatment followed by neutralization. The native and treated proteins, amaranth (A and TA), soybean (S and TS) and the 1:1 mixture (M and TM) were studied. The structural characteristics and surface tension and foaming properties of the proteins were analyzed.

The acid-neutralization treatment caused structural modifications on all the proteins. The soybean proteins suffered some conformational changes and dissociation whereas the amaranth proteins were partially hydrolyzed by an endogenous aspartic protease, activated at acid pH. M showed the equivalent presence of S and A proteins, but TM, presented characteristics more similar to the TA proteins suggesting that the amaranth protease acted on the soybean proteins.

The acid treatment did not modify the S tensioactivity while TA and TM increased their tensioactivity compared to A and M. Amaranth proteins showed to be faster and more efficient than S in decreasing the surface tension, and present the higher velocity of foam formation. The acid treatment improved the foam formation capacity of all samples. Foam stabilization was also enhanced by the acid treatment, though in this case S proteins were better foam stabilizers than A. Although M showed an intermediate behavior between S and A, the TM showed a foam stability nearer the TS.

The mixture of amaranth and soybean proteins subjected to acid treatment make up an ingredient with improved surface and foam properties compared with its non treated components.

1. Introduction

Among seed proteins, those of soybean and amaranth stand out because of their high nutritional quality. Their amino acid compositions are close to the human diet requirements and are complementary (Bressani, 1994; Liu, 2000). Concerning their food functionality, it was reported that soybean proteins exhibit high functional properties compared to other plant proteins (Hettiarachchy & Kalapathy, 1998, chap. 6; Zayas, 1997). On the other hand, more recently the functional properties of amaranth proteins have begun to be studied, and it has been informed that although they show a lower solubility than soy proteins, they present good functional properties (Abugoch, Martinez & Añón, 2010; Avanza, Puppo, & Añón, 2005; Bejano-Luján, Lopez da Cunha & Netto, 2010; Fidantsi & Doxastakis, 2001; Mahajan & Dua, 2002; Marcone & Kakuda, 1999; Ventureira, Martinez & Añón, 2010). On the basis of these characteristics it seems reasonable to propose the use of these two protein sources to prepare a mixed food ingredient, which is expected to present excellent functional and nutritional qualities.

Many foods, most of them composed of foams and/or emulsions, require ingredients with good surface properties. The capacity of a protein to form and stabilize foams and emulsions depends on its structural characteristics and its physicochemical properties; e.g., a high solubility favors foaming and emulsifying properties. Flexible protein molecules showing an important surface hydrophobicity present a higher foaming capacity, whereas foam stability depends on the protein capacity to develop intermolecular bonds forming a viscoelastic film (Damodaran, 1997; Utsumi, Matsumura, & Mori, 1997).

Many plant storage proteins present a packed and scarcely flexible globular conformation that limits their foaming capacity, which may be improved by means of structural modifications. Chemical, physical and enzymatic modifications have been shown to improve functional properties. Amaranth proteins have turned more soluble with better foaming properties upon enzymatic hydrolysis (Condes, Scilingo, & Añón, 2009; Scilingo, Molina Ortiz, Martinez, & Añón, 2002), and chemical and enzymatic treatments have been shown to increase the foaming capacity of soybean

* Corresponding author. Tel./fax: +54 221 4249287.
E-mail address: mca@biol.unlp.edu.ar (M.C. Añón).

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proteins (Molina Ortiz & Wagner, 2002; Wagner & Gueguen, 1999a,b).

HCl addition is an easy treatment to reduce the pH of protein solutions without adding other chemicals but Cl^-; upon this treatment the protein molecules unfold, expose more hydrophobic patches and become more flexible. This favors their adsorption in the interface and decreases the surface tension. Although these characteristics favor foam formation, foam stability is poor when completely unfolded proteins are used. Partial unfolding may be reached by mild acid treatments or by acid treatment followed by neutralization. It was demonstrated that after this pH-shifting process, soy proteins adopted a molten globule-like conformation that led to a markedly improved emulsifying activity and emulsion stability (Jiang, Chen, & Xiong, 2009).

The purpose of this work was to obtain a food ingredient with foaming properties, composed of soybean and amaranth proteins. Considering the possibility of hybrid structures formation with better functionality, the mixture of proteins was subjected to an acid treatment followed by neutralization. The structural and surface properties of the mixture and its isolated components were studied.

2. Materials and methods

2.1. Flours

Amaranth flour was prepared from seeds of *Amaranthus hypochondriacus* (Mercado commercial cultivar) kindly provided by Estación Experimental del Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP), Chapingo, México. They were ground in an Udy mill (UDY Corp., USA) equipped with a 1 mm mesh and screened through a 10 xx mesh (92 μm) (Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Argentina). The flour was defatted with hexane (10% w v^{-1} suspension) at room temperature during 24 h, under continuous stirring during the first 5 h. The defatted flour was dried at room temperature and stored at 4 °C until used.

Commercially defatted soybean flours were donated by Sanbra S. A. (Brazil).

2.2. Protein isolates

The amaranth isolate was prepared according to Martínez and Añón (1996). The flour was dispersed in water in a 1:10 w v^{-1} ratio and the pH was adjusted to 9.0 by adding 2 M NaOH. The dispersion was stirred during 1 h and then centrifuged at 9000 *g* for 20 min at 10 °C. Proteins were precipitated by adjusting the supernatant to pH 5.0 with 2 M HCl, and were separated by centrifugation at 9000 *g* for 20 min at 4 °C. The pellet was dispersed in a small volume of water, neutralized with 0.1 M NaOH, and freeze-dried. The soybean protein isolate was prepared according to the method described by Petruccelli and Añón (1994). A 1:10 w v^{-1} dispersion of flour in water was adjusted to pH 8.0 with 2 N NaOH and stirred for 1 h. Then it was centrifuged at 10,000 *g* for 30 min at 4 °C, and the supernatant was adjusted to pH 4.5 to precipitate proteins. After centrifugation at 10,000 *g* for 20 min at 4 °C the precipitate was dissolved in water, neutralized and freeze-dried.

2.3. Acid treatment of the isolates

Each soybean and amaranth isolate and a 1:1 mixture of both isolates were dispersed in 0.01 M HCl at a concentration of 10 mg mL^{-1}, and the pH was adjusted to 2.0 with 2 M HCl. After stirring at room temperature for 3 h dispersions were diluted to 1 mg mL^{-1} with 35 mM phosphate buffer pH 7.5, 0.4 M NaCl (buffer A). These preparations were stirred for 3 h at room temperature and stored for 18 h at 4 °C before their use in different assays. The protein content of the isolates, as determined by the Kjeldahl method, was 83.1% ± 0.5 (db) amaranth (factor 5.85, Becker et al., 1981) and 85.2% ± 0.3 (db) soybean (factor 5.71, Wilson, 1995, chap. 22). The samples used for the assays were: native soybean isolate (S); amaranth isolate (A); 1:1 mixture of native soybean and amaranth isolates (M); treated soybean isolate (TS); treated amaranth protein isolate (TA); treated 1:1 mixture of soybean and amaranth isolates (TM).

2.4. Solubility

The native and treated samples were dispersed in buffer A (1 mg mL^{-1}) and stirred for 1 h at 25 °C. These dispersions and aliquots of diluted (1 mg mL^{-1}) treated and native samples were centrifuged at 15,000 *g* during 15 min at 20 °C. Protein content in the supernatant (P_s) was determined by the Lowry method and this value was used to calculate percent protein solubility in relation to the initial protein content in the sample (P_{in}), as follows:

$$S\% = P_s \times 100/P_{in}$$

Determination of solubility of the treated samples was carried out at the end of the acid treatment detailed before.

2.5. Gel filtration chromatography (FPLC)

Native and treated samples dissolved in buffer A were analyzed in a Pharmacia LKB, FPLC System (Uppsala, Sweden). Samples (200 μL) were injected in a Superose 6B HR 10/30 column and eluted with buffer A. The optical density at 280 nm was recorded. The column was calibrated with blue dextran (V_0), thyroglobulin (669 kDa). α-amylase (200 kDa), alcohol dehydrogenase (150 kDa), albumin (66 kDa), cytochrome C (12.4 kDa) and aprotinin (6.5 kDa). The calibration curve obtained from duplicate measurements was:

$$\log MM = A - (B \times K_{AV}) \quad \text{and} \quad K_{AV} = (V_e - V_0)/(V_T - V_0)$$

where MM is the molecular mass in kDa; V_e is the elution volume in mL; V_T is the total volume of the column (25 mL) and V_0 is the void volume. Curves were processed and data were evaluated using Pharmacia AB, FPLC director and FPLC assistant software.

2.6. Electrophoresis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed in minislabs (Bio-Rad Mini Protean II Model) according to Laemmli's method (1970). Runs were carried out with 12% (w v^{-1}) acrylamide gels and the following continuous buffer system: 0.375 M Tris–HCl, pH 8.8, 0.1% (w v^{-1}) SDS for the separating gel; 0.025 M Tris–HCl, 0.192 M glycine and 0.1% (w v^{-1}) SDS, pH 8.3 for the running buffer, and 0.125 M Tris–HCl, pH 6.8, 20% (v v^{-1}) glycerol, 1% (w v^{-1}) SDS, and 0.05% (w v^{-1}) bromophenol blue as sample buffer. For runs under reducing conditions the sample buffer contained 5% 2-mercaptoethanol (2-ME) and samples were heated for 1 min in a boiling-water bath. Samples containing 40–50 μg of protein were loaded. The following protein molecular mass standards were used: phosphorylase b (94 kDa); bovine serum albumin (67 kDa); ovalbumin (45 kDa); carbonic anhydrase (30 kDa); trypsin inhibitor (20.1 kDa); a-lactalbumin (14.4 kDa). Gels were fixed and stained with Coomassie Brilliant Blue Stain or Silver Stain as indicated in the figures. Gels images were scanned.

2.7. Differential scanning calorimetry

DSC measurements were performed in a TA Q100 (TA-Instruments, USA) calorimeter. The equipment was calibrated at a heating

rate of 10 °C min^{-1} by using indium, lauric acid, and stearic acid (p.a.) as standards.

Samples were prepared by dispersing freeze-dried isolates in buffer A (20% w w^{-1}). The treated isolates were previously dialyzed against distilled water, freeze-dried and then were dispersing in buffer A. Hermetically sealed aluminum pans were prepared to contain 15–22 mg of sample dispersion, and a double empty pan was employed as reference. Capsules were heated from 40 to 120 °C at a rate of 10 °C min^{-1}. After each run, the pans were punctured, and their dry-matter content was determined by leaving the pans overnight in an oven at 105 °C. The denaturation temperature (Td) and enthalpy of transition (ΔH) were obtained by analyzing the thermograms with the Universal Analysis 2000 Software. The ΔH value was calculated from the area below the transition peaks.

2.8. Fluorescence spectroscopy

The intrinsic fluorescence of the samples was determined at 25 °C in buffer A with a Perkin–Elmer LS 50B fluorescence spectrophotometer at an excitation wavelength of 290 nm (slit width, 3 nm), an emission wavelength of 310–550 nm (slit width, 3 nm), and a scanning speed of 300 nm min^{-1}. Samples at concentrations between 0.01 and 0.03 mg mL^{-1} were obtained by dilution of the initial 1 mg mL^{-1} protein solution (determined by the Lowry method).

Ten spectra (two from each of five separate assays) for each protein component were averaged and used for analysis.

2.9. Surface hydrophobicity

The surface hydrophobicity (H_0) of the protein samples was measured using the fluorescent probe ammonium 8-(anilino) naftalene-sulfonate, ANS (Aldrich Chemical Co.) according to the method of Cardamone and Puri (1992). Samples were dissolved in buffer A, centrifuged (10,000 *g*, 20 min at 20 °C) and diluted to obtain a protein concentration of approximately 0.12 mg mL^{-1}. Increasing volumes of a 10 mM ANS solution were added to the protein solution so as to cover a range of ANS concentration from 0 to 100 μM. At each ANS addition the fluorescence intensity was measured with a fluorescence spectrophotometer (LS 50B model, Perkin–Elmer Corp.). The mixture was excited at 350 nm and the emission spectrum (450–540 nm) was read. A blank mixture of buffer and the different ANS concentrations was also run to obtain ΔFI, the increase in fluorescence as a result of ANS binding. The ΔFI at 470 nm (the maximum emission of an ANS–protein complex) was finally plotted against the ANS concentration (μM) and the data adjusted to the following equation:

$$\Delta FI = A \times ANS/(B + ANS)$$

A and *B* values were obtained using ORIGIN Pro 8 software (OriginLab Co; EE.UU). "*A*" is the maximum fluorescence intensity for the protein concentration used when the surface protein is completely saturated with ANS (ΔFI max).

Surface hydrophobicity, H_0, is calculated as:

$$H_0 = A/PC$$

where PC is the protein concentration in the tube expressed in mg mL^{-1}.

2.10. Drop tensiometry

The surface tension was measured with a drop volume tensiometer (LAUDA TVT2, Lauda DR. R. WOBSER GMBH and Co., Germany), using the dynamic mode. The samples were suspended in buffer A, and stirred for 1 h at room temperature. Then they were centrifuged at 1000 *g* for 5 min at 20 °C. The supernatant was separated and adjusted to a protein content of 0.5 mg mL^{-1}. In the dynamic mode, the pendant drop is made at different rates and the surface tension is calculated at each velocity by the volume of the drop when it detaches from the capillary. This allows obtaining a plot of the interfacial tension as a function of the age of the interface. Two independent experiments were performed for each sample varying the rate of drop formation, with 3 measures taken for each rate. The rates of drop formation were set to cover a range between 1.25 μL s^{-1} and 12.5 μL s^{-1}. The assays were performed at 20 ± 0.5 °C.

The tension value of the buffer used was 71.3 ± 0.3 mN m^{-1}.

Experimental data – surface tension (γ) and the time elapsed until the detaching of the drop from the capillary (tD) – were used to calculate the linear regressions of γ vs tD $-1/2$ plots. The intercept gives an estimation of the value of the surface tension at the equilibrium.

2.11. Foaming properties

Foaming properties were evaluated by conductimetry using the method and apparatus developed by Loisel & Popineau (1993). Foam was formed using a graded glass column having a fritted glass disk (G4 type, 5–15 μm) at the bottom. N_2 gas was bubbled at a flow rate of 40 mL min^{-1} during 1 min through 6 mL of 1 mg mL^{-1} of native or treated isolates suspended in buffer A. The level of the solution as a function of the time was measured by conductimetry. The maximum volume of liquid incorporated into the foam (V_{max}, mL), the rate of liquid incorporation to the foam at the first 5 s (v_{in}, mL min^{-1}), and the time for draining half of the liquid incorporated in the foam after stopping the bubbling ($t_{0.5}$ s) were determined. Determinations were performed by triplicate at room temperature.

2.12. Statistical treatment

The least significant difference (LSD) test (after analysis of variance, ANOVA) was used to identify pairwise differences between means. Significance was determined at $p < 0.05$.

3. Results and discussion

3.1. Structure of treated and untreated (native) isolates

The six protein isolates were analyzed by electrophoresis (Fig. 1a).

The amaranth isolate pattern shows the presence of globulin polypeptides of 56, 45, 30 and 20 kDa, albumin and small globulin polypeptides of low molecular weight (Fig. 1a, arrows). As compared to the A isolate the TA contains a higher amount of polypeptides of low molecular weight. The S and TS patterns are identical, showing the presence of the β-conglycinin α, α′ and β polypeptides, and glycinin A and B polypeptides (Fig. 1a). The profile of M exhibited a combination of the bands present in S and A profiles, and the same relation was observed between the TM profile and the profiles of TS and TA.

The TA profile suggested that the amaranth isolate was hydrolyzed during the acid treatment. The presence of an endogenous protease activated in acid medium was confirmed by several tests, some of them are shown in Fig. 1b and c. In Fig. 1b it is demonstrated the absence of hydrolysis when the isolate was previously subjected to a heat treatment and in Fig. 1c the inhibition by pestatine suggests that an aspartic protease was present in the isolate. Aspartic proteases with optimum pH around pH 3.0 were described in barley and cacao seeds (Guillotea, Lalo, Michaux, Bucheli, &

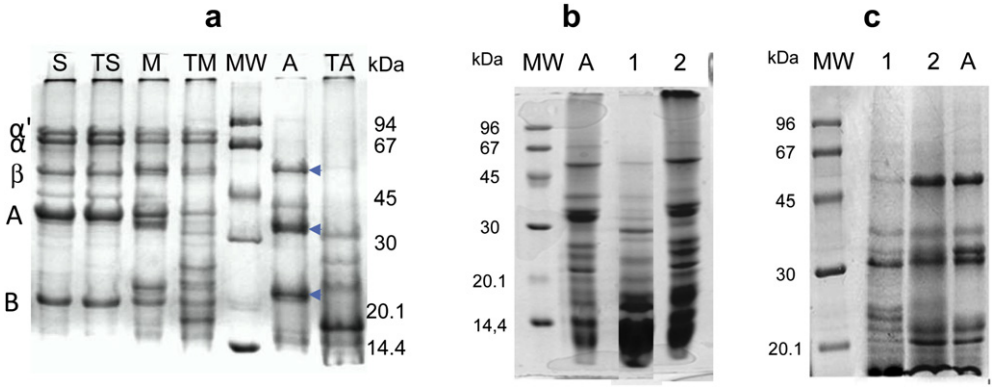

Fig. 1. SDS-PAGE in reducing conditions. (a) Soybean, mixture and amaranth isolates, S, M and A: and the corresponding treated samples TS, TM and TA. Soybean polypeptides are indicated on the left, and amaranth polypeptides are indicated with arrows. (b) Presence of a protease in the amaranth isolate. Amaranth untreated isolate, lane A; amaranth isolate treated 3 h at pH 2, lane 1; amaranth isolate heated 10 min at 90 °C followed by treated 3 h at pH 2, lane 2. Acrylamide 13.5% gel. (c) Presence of a protease in the amaranth isolate. Amaranth isolate treated 3 h at pH 2, 1; amaranth isolate treated 3 h at pH 2 in the presence of pepstatine A, 2; amaranth untreated isolate, A. Acrylamide 12% gel. Molecular weight standards, MW; standard molecular masses, kDa.

McCarthy, 2005; Runeberg-Roos, Tormakangas, & Ostman, 1991) probably located in vacuoles (Mutlu, Chen, Reddy, & Gal, 1999; Tormakangas, Kervinen, Ostman, & Teeri, 1994).

The structural characteristics of the isolates were studied by DSC. As shown in Fig. 2 the S and A isolates showed two endotherms – at 88.4 ± 0.3 °C and 104.2 ± 0.2 °C for S and 75.5 ± 0.2 °C and 101.8 ± 0.3 °C for A – corresponding to the globulins. The larger denaturation enthalpy of soybean proteins indicates that their molecules are more structured, probably containing a higher number of hydrogen bonds. The M isolate also presented two endotherms whose maximum deflection temperatures are intermediate between those indicated above – 86.6 ± 0.4 °C and 103.6 ± 0.3 °C. This fact may be explained by an interaction between molecules from the two isolates, which may have generated a more stable structure. The TA thermograms showed no endotherm indicating that the molecules were unfolded. In contrast, the TS thermogram presented two small endotherms – at 89.0 ± 1.5 °C y 104.4 ± 0.4 °C – with lower enthalpy suggesting that some molecules with a small degree of folding are present. A unique small endotherm at 86.1 ± 1.1 °C was detected in TM samples.

The chromatographic and SDS-PAGE analyses of the soluble fractions in buffer A showed that all the β-conglycinin and glycinin components of the soybean isolate were present as aggregates

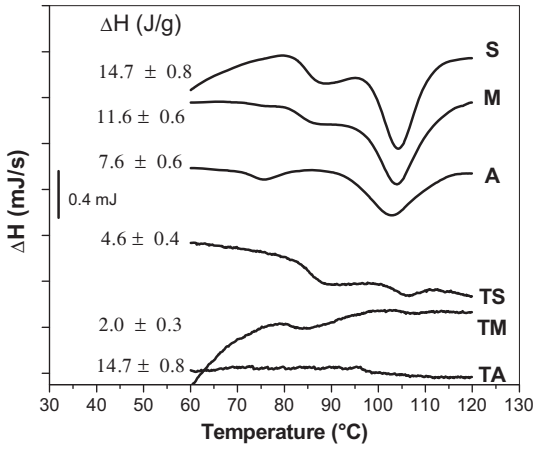

Fig. 2. DSC thermograms of soybean, mixture and amaranth isolates, S, M and A: and the corresponding treated samples TS, TM and TA. On the left the denaturation enthalpies (Δ*H*) are indicated.

larger than 500 kDa, as molecules of around 400 kDa (Fig. 3a, fractions S, 1–5) and lower molecular weight species. On the other hand the soluble fraction of the A isolate contained few molecules of globulins (Fig. 3c, peaks 1–3) but presented a larger amount of low molecular weight molecules corresponding to the small globulin and albumins.

As revealed by the SDS-PAGE pattern of TS (Fig. 3a), all the species of the S isolate were present in the soluble fraction of the treated isolate, but the chromatographic profile, with a higher proportion of low molecular weight species, indicated that some of the molecules were dissociated. The soluble fraction of the TA isolate (Fig. 3c) was composed of low molecular weight molecules integrated by small polypeptides, most of them hydrolysis products. Analyses of the M isolate (Fig. 3b) showed that soybean and amaranth species contributed to the mixture in both the untreated and treated preparations. Apparently TM had a higher proportion of smaller molecules than would be expected if TA and TS contributed equally to the mix.

The structure of the soluble fraction of the isolates was studied by fluorescence spectroscopy (Fig. 4). The spectrum of the S sample presented a maximum at a wavelength (λ_{max}) rather lower than that of the A sample (Fig. 4, inset), suggesting that tryptophans of amaranth proteins could be more exposed to the polar medium (Permyakov, 1993). The TA sample showed a fluorescence spectrum with a lower FI_{max} and a higher λ_{max} than that of the untreated sample, in agreement with the chromatographic results showing small polypeptides which would be exposing their tryptophans to a more polar environment. On the contrary, the TS spectrum presented minor changes relative to that of the untreated sample (Fig. 4 inset) which is in accordance with the partial dissociation detected by DSC and chromatography suggesting that the molecules that were not dissociated maintained their structure. Interestingly the TM spectrum presented characteristics (FI_{max} and λ_{max}) nearer to the spectrum of treated amaranth (TA) probably due to the action of the endogenous protease on soybean proteins besides A ones.

The surface hydrophobicity values of the samples are informed in Table 1. The S isolate presented a higher surface hydrophobicity than the A isolate, whereas the M sample showed an intermediate value. After treatment, the soybean proteins exposed a higher number of hydrophobic patches probably corresponding to the dissociated molecules. On the other hand the surface hydrophobicity of the TA proteins was lower than that of proteins in the untreated sample which may be explained considering that the

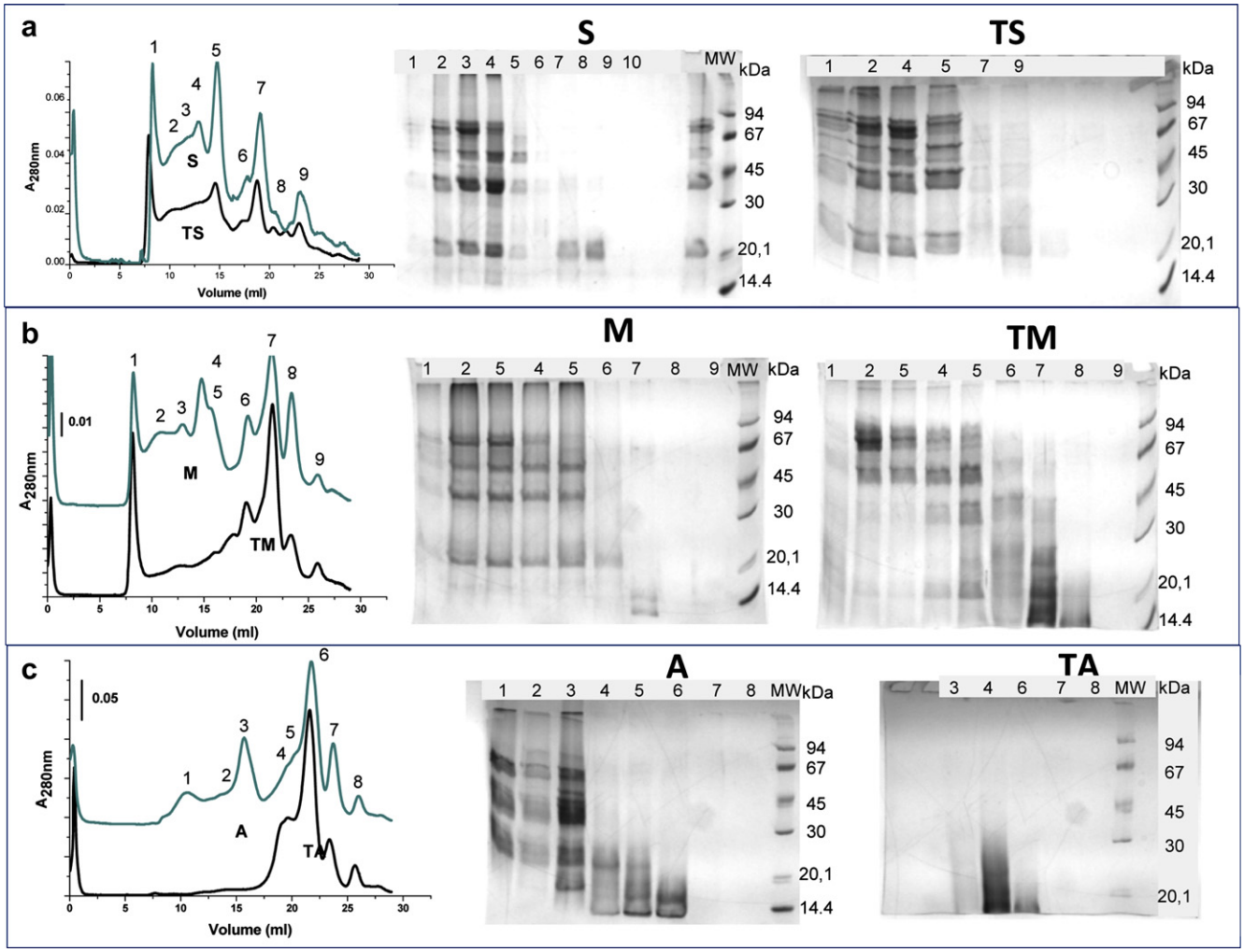

Fig. 3. FPLC and SDS-PAGE of samples soluble in buffer A, (a) Soybean, S and treated soybean, TS, isolates; (b) Mixture, M and treated mixture, TM isolates; (c) Amaranth, A and treated amaranth, TA, isolates. In the SDS-PAGE gels the numbers of the lanes are the peak fractions of the corresponding chromatogram. Molecular weight standards, MW; standard molecular masses, kDa.

dissociated hydrolyzed peptides did not expose patches large enough to link the ANS hydrophobic probe. The hydrophobicity values of the TM sample revealed an equal contribution of proteins from both isolates.

3.2. Surface tension

Fig. 5 shows the decrease in surface tension with time. S proteins were the ones with the lowest reduction of surface tension during the assay. The curves corresponding to untreated A and M are almost overlapped, probably because amaranth proteins dominate over soy proteins in the behavior of M. Acid treatment did not seem to affect the tensioactive activity of S as much as it did with other samples, since TS curves did not differ markedly of S curve and even overlapped at times lower than 10 s. On the contrary, in the A and M samples the acid treatment and subsequent neutralization improved the tensioactive capacity of protein isolates, since the velocity of diminution of surface tension increased.

It must be kept in mind that the dynamic mode was used to measure interfacial tension. When this mode is applied for the maximal velocity of drop formation (12.5 μL min^{-1}), a shorter time measured for a given sample indicates that the drop detached with a lower volume. It can be deducted that the velocity of diminution of the surface tension increases as the time for drop detachment decreases. Surface tension values measured with the tensiometer at the time of detachment of the first drop and values of ordinate at the origin corresponding to the curve γ values vs $t^{1/2}$ – which represent the magnitude of surface tension at infinite time – are shown in Table 1. As compared with untreated samples, acid-treated samples exhibited a reduction of surface tension in all cases (Ruíz-Henestrosa, Sánchez, Pedroche, Millán, & Rodríguez Patino, 2009; Ward & Tordai, 1946). Surface tension at infinite time was not modified by acid treatment and subsequent neutralization in the case of soy proteins, but was reduced in the case of amaranth proteins and the isolates mixture. Surface tension reduction is probably due to the smaller size of proteins after treatment, caused by either subunits dissociation or by the hydrolysis of TA and TM proteins due to the hydrolytic factor that acts at acid pH.

The tensioactive capacity of amaranth proteins was higher than that of soy proteins both before and after treatment. It is interesting to note that for the mixture of isolates, the values of tension to infinite time are not the average of soy and amaranth values; conversely, a synergism exists between both species making such values to diminish as much as amaranth ones. This may be due to the fact that in these experiments only amaranth proteins reach the

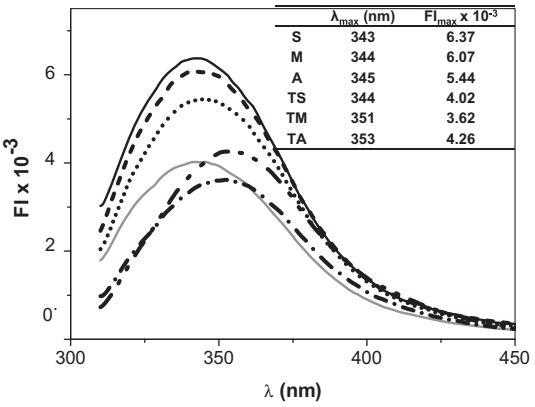

Fig. 4. Intrinsic fluorescence spectra of isolates: soybean S (—), treated soybean, TS (·····), mixture M (––), treated mixture TM (– · – · -), amaranth A (·····) treated amaranth TA (— · · —). Inset, the maximum fluorescence intensity (FI_{max}) and the wavelength at FI_{max} (λ_{max}) values of the samples are shown.

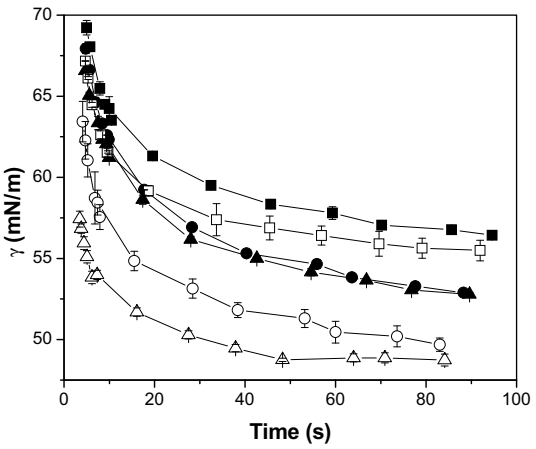

Fig. 5. Surface tension vs time during drop formation with samples in buffer A. Untreated isolates; soybean, S (-■-); mixture, M (-●-); amaranth, A (-▲-). Treated isolates, soybean, TS (-□-); mixture, TM (-○-), amaranth, TA (-△-).

interface due to their higher adsorption and repositioning velocity, or they may be so closer that the fact that the intimate contact between protein species results in interactions that allow A proteins to be more efficient as tension reducers.

3.3. Foaming properties

3.3.1. Initial foaming velocity

The most critical point in foam formation is the rapid reduction of the interfacial tension of the area created by the tensioactive agent. For the samples under study, data regarding initial foaming velocity are shown in Fig. 6, which clearly shows in all cases an improvement of this parameter with acid treatment. Graham and Phillips (1976) demonstrated that the most important factor for the foaming capacity of a protein solution is the velocity at which the protein can reduce the interfacial tension while a larger surface is being created due to stirring or bubbling. Results of surface tension diminution showed that amaranth proteins are more rapid and efficient in this respect, which agrees with results of initial foaming velocity. Fig. 6a shows that initial foaming velocity value of A is somehow greater than S and M, and the treated sample showed larger values than the non treated ones being not different between each other.

In order to form foams the protein must reach the interface quickly to accommodate and change its conformation (Damodaran, 1994, chap. 1); therefore, the protein must be in soluble form (Walstra, 1989; Yalçin & Çelik, 2007). Under the assay conditions used, (pH 7.5, μ 0.5), A and TA samples exhibited the lowest solubility (45% and 43%, respectively), followed by M and TM (68% and 60%, respectively) and finally by S and TS (78% and 87%, respectively). No correlation was observed between the foaming velocity and the solubility of the different samples. While, A and TA exhibited the lowest solubility, their foaming velocities were equal (TA vs TS and TM) or higher (A vs S and M) than those of other samples. This fact evidences that, in spite of their lower concentration, amaranth soluble proteins have a greater foaming capacity than soy proteins.

Table 1
Surface hydrophobicity and surface tension parameters.

	H_0[a]	$\gamma_{t\infty}$ (mN/m)[b]	$\gamma_{1st\ drop}$ (mN/m)[c]
S	3599 ± 71ab	69.54 ± 0.45a	52.8 ± 0.4a
M	3268 ± 168b	68.15 ± 0.30b	48.6 ± 0.1b
A	2530 ± 4c	66.65 ± 0.12c	48.9 ± 0.0b
TS	4018 ± 93a	67.19 ± 0.02c	52.8 ± 0.9a
TM	2156 ± 222c	64.33 ± 1.25d	46.6 ± 0.2c
TA	960 ± 57d	57.78 ± 0.49e	46.2 ± 0.2c

Values are means ± standard deviation. In each column different letters indicate significantly different values ($p < 0.05$).
[a] Surface hydrophobicity.
[b] Surface tension at infinite time.
[c] Surface tension at the time of the first drop detachment.

3.3.2. Stability of foams

Proteins are good foam stabilizers when their molecules can form a viscoelastic film in the interface through intermolecular interactions (Damodaran, 1997). The half life of foams formed with the different samples under study is shown in Fig. 6b. It can be observed that in all the cases the acid treatment results in foams with much higher stability. Among native samples, S exhibited the highest stability and A the lowest, with M exhibiting an intermediate behavior. The behavior of the TM samples is remarkable among acid-treated samples. Synergic effects between soy and amaranth proteins took place after treatment, so that mean times were not the result of an additive effect between individual species but were instead higher than their average. As a consequence, no significant differences were observed between the stability values of TS and TM proteins, which were the most stable emulsions among those under study.

It is evident that changes that occur in the native structure of proteins due to the exposure to acid pH favor foam stabilization. As demonstrated by DSC, FPLC, and PAGE, proteins are less structured after acid treatment and contain, in the case of TA and TM samples, polypeptides that suffered some degree of hydrolysis. Probably in this case the fact that the protein has a more extended and flexible structure allows rearrangements and interactions between protein molecules in the surface film to occur more rapidly and easily, this being a necessary condition for foam stabilization. It must be kept in mind that foams are systems that destabilize quickly due to gravity and surface tension. An increasing complexity of the protein structure slows down the tensioactive capacity of proteins. According to Xu and Damodaran (1994), changes in the tertiary structure of proteins are very important for the modification of foam properties, a fact that was observed in the present study for all the samples after the acid treatment of protein isolates.

Stability results influence on those of initial foaming velocity. If one takes into account that as foam is forming the existing one is simultaneously being destabilized, and that the stability of the different samples is lower for TA foams than for TS or TM ones, it

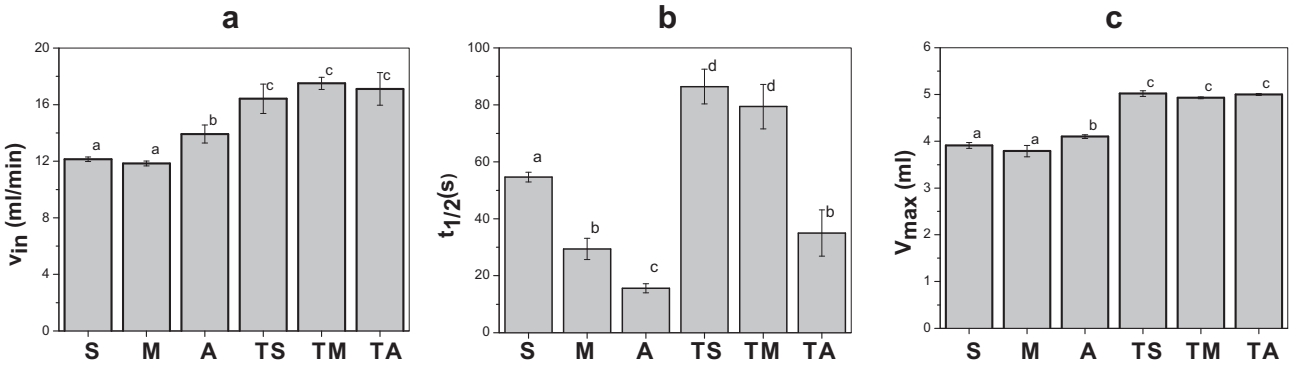

Fig. 6. (a) Initial foaming velocity of samples. (b) Half life time ($t_{1/2}$ s) of foams prepared with the samples. (c) Maximum volume of liquid incorporated into the foams prepared with samples. Samples: untreated isolates soybean, S; mixture, M and amaranth, A; treated isolates, soybean, TS; mixture, TM and amaranth, TA.

can be concluded that foaming is more rapid when it is done from TA suspensions. Despite the fact that TA has the same initial foaming velocity than TM and TS, it destabilizes more rapidly making necessary to form more foam to measure equal values using this technique.

3.3.3. *Maximal volume of fluid incorporated by the foam*

As shown in Fig. 6c, acid treatment and subsequent neutralization increased in all cases the values of maximal fluid volume incorporated by the foam. Taking into account that the magnitude of such volumes is influenced by both initial foaming velocity and foam stability at short term, these results agree with the later two parameters. The volume of dispersion fluid incorporated by the foam increased after acid treatment. The reduced long-term stability of amaranth proteins is compensated by their better foaming capacity, allowing to reach significantly higher maximal volumes of incorporated fluid, in the case of the untreated protein (S, A and M), and equal volumes in the case of the treated protein (TS, TA and TM). Graham and Phillips (1976) related a good foaming capacity with proteins with higher flexibility. These authors also indicated that highly ordered and less flexible globular proteins have more difficulty to rearrange in the interface, resulting in a lower foaming capacity. In the present study, acid pH exposure disorganized the globular structure, which was not recovered after raising the pH to almost neutral values, thus allowing to improve their foaming capacity.

We can conclude that the present results show that the mixture of amaranth and soybean proteins subjected to acid treatment make up an ingredient with improved surface and foam properties compared with its non treated components.

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