Effects of Thermal Treatment of Soy Protein Isolate on the Characteristics and Structure–Function Relationship of Soluble and Insoluble Fractions

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Aqueous dispersions (5–15% w/w) of native soy protein isolate were thermally treated at 80 and 100 °C for 30 min. At 100 °C, both 7S and 11S proteins were totally denatured, while at 80 °C, the 7S protein was totally denatured but the 11S protein was not, the denaturation degree of which was only partial and dependent on the extent of isolate denaturation. The different behaviors were reflected in the degree of aggregation, which in turn determined the yields and structural characteristics (denaturation degree, molecular weight distribution, superficial hydrophobicity, Mg^{2+} induced aggregation) of soluble and insoluble fraction obtained from treated isolates. A study was then performed on the effects of such structural changes on some functional properties (solubility, water imbibing, gelling and foaming properties) of soluble and insoluble fractions and, consequently, those of the total isolate.

Keywords: *Soy proteins; thermal treatments; structure–function relationship*

INTRODUCTION

Soybeans constitute the most important vegetable source of protein ingredients for food formulation. Isolates are the most refined form of soybean proteins, and they must have suitable functional properties so as to make the formulation successful, particularly with regard to consumer acceptability (Kinsella, 1979). The functional properties of soy isolates reflect the composition and structure of their major components: 11S (glycinin) and 7S (β-conglycinin) globulins (Kinsella et al., 1985; Rhee, 1994). The protein structure can be modified by different treatments to improve specific functional properties, heating being one of the more frequently used for that purpose (Yamauchi et al., 1991). Many workers have studied the effect of heat treatment under different conditions (temperature, time, protein concentration, pH, ionic strength) on functional properties such as solubility, water absorption, gelation, emulsification, and foaming (Hermansson, 1978; Kinsella, 1979; Petruccelli and Añón, 1994a,b). It has been demonstrated that the functionality depends basically on the degree of dissociation, denaturation, and aggregation of the 7S and 11S globulins (Utsumi et al., 1984; Wagner and Añón, 1990; Arrese et al., 1991). It is also known that soy protein isolates (whether commercial or prepared in laboratory) show varying percentages of soluble and insoluble proteins. Studies previously performed in our laboratory on commercial soy isolates showed that some functional properties are basically determined by the type and content of the insoluble fraction (Sorgentini et al., 1991).

In the present work we studied the composition, distribution, and structural characteristics as well as the functional properties of proteins present in the soluble and insoluble fractions of native and thermally modified isolates to determine the influence of thermal treatment on the functional properties of the total isolate.

MATERIALS AND METHODS

Samples. *Native Soy Protein Isolate.* Native isolate was prepared from defatted soybean flour (Sanbra S.A., Brazil) by extraction for 2 h at room temperature with water adjusted at pH 8.0 with 2 N NaOH (water/flour ratio 10:1) and centrifugation at 5600 rpm (GSA rotor, Sorvall RC 5B refrigerated Superspeed Centrifuge) for 15 min at 20 °C. The extract was adjusted to pH 4.5 with 1 N HCl, kept for 2 h at 4 °C, and centrifuged at 6500 rpm (Sorvall GSA rotor) for 20 min at 4 °C. The precipitate was washed with water, resolubilized in water by neutralization to pH 8.0 with 2 N NaOH at room temperature, and lyophilized (Thermovac Industries Corp. freeze-dryer). The 7S/11S ratio of native isolate was 0.68 ± 0.01 (determined from SDS–PAGE).

Modified Soy Protein Isolates. Aqueous dispersions of native soy isolate (5%, 8%, 11%, 13%, and 15% w/w) were prepared by magnetic stirring at room temperature. Heat-treated isolates were prepared by heating dispersions at 80 and 100 °C for 30 min and then cooling overnight at 4 °C. Viscous dispersions (at 5% isolate concentration) or gels (at concentrations equal to or greater than 8%) were obtained. Gels were disrupted with a glass rod. Part of the viscous dispersions and disrupted gels were diluted with water to approximately 4% w/w isolate concentration, homogenized (1500 rpm for 1 min, Virtis blender), and lyophilized.

Soluble and Insoluble Fractions. The remaining part of the aqueous dispersions of native and modified soy isolates (4% w/w, pH 7.0) were centrifuged at 6500 rpm for 30 min (Sorvall GSA rotor). Supernatant and precipitate (soluble and insoluble fractions) were separated and lyophilized. The yield of soluble and insoluble fractions was calculated as a percentage (w/w) of the respective total isolate.

All lyophilized isolates and fractions were ground in a mortar to obtain a fine powder.

Differential Scanning Calorimetry (DSC). Samples (15–20 mg) of 20% dispersions in water were hermetically

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sealed in aluminum pans. An empty double pan was used as reference. The samples were analyzed at 10 °C/min in a DuPont Model 910 calorimeter attached to a Hewlett-Packard 7046B recorder. The areas under the endotherm curves were measured with a Morphomat 34 Zeiss image analyzer, and the corresponding enthalpies of thermal denaturation (ΔH in Joules per gram of dry matter) were calculated. All of the assays were performed as previously described (Wagner and Añón, 1990) and repeated at least twice.

Surface Hydrophobicity (S_0). S_0 was determined by the hydrophobicity fluorescence probe 1-anilino-8-naphthalenesulfonate (ANS). Measurements were performed according to the method of Kato and Nakai (1980), in the absence of SDS. Protein dispersions (1 mg/mL for soluble fractions and 5–10 mg/mL for insoluble fractions) in 0.01 M phosphate buffer, pH 7.0, were stirred for 2 h at 20 °C and centrifuged at 6500 rpm (Sorvall SS34 rotor) for 20 min. Protein concentration in the supernatants was determined according to the method of Lowry et al. (1951). Each supernatant was serially diluted with the same buffer to obtain protein concentrations ranging from 0.1 to 0.0005 mg/mL. Then 40 μL of ANS (8.0 mM in 0.1 M phosphate buffer, pH 7.0, solution) was added to 2 mL of sample. Fluorescence intensity (FI) was measured with a Perkin-Elmer 2000 fluorescence spectrometer, at wavelengths of 365 nm (excitation) and 484 nm (emission). The initial slope (S_0) of FI vs protein concentration plot (calculated by linear regression analysis) was used as an index of the protein hydrophobicity.

Electrophoresis (SDS–PAGE). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was carried out after reduction of the protein by 2-mercaptoethanol (5% v/v). Runs were performed in 5–15% polyacrylmide gradient gels in a Bio-Rad mini-Protean electrophoresis cell at a constant voltage (200 V). A continuous and dissociating buffer system, containing 0.375 M Tris-HCl, pH 8.8, and 0.1% SDS for the separating gel and 0.025 M Tris-HCl, 0.192 M Gly, and 0.1% SDS, pH 8.3, for the run buffer, was used. Gel slabs were fixed and stained simultaneously in a solution of methanol, acetic acid, and water (5:5:2) and 0.1% Coomassie Brillant Blue R-250. Molecular weights of the proteins were estimated by means of the MW-SDS-70L Sigma kit (α-lactalbumin, 14 200; trypsin inhibitor, 20 100; trypsinogen, 24 000; carbonic anhydrase, 29 000; glyceraldehyde-3-phosphate dehydrogenase, 36 000; ovoalbumin, 45 000; bovine albumin, 66 000). Densitograms were obtained by means of a TLC scanning CS-910 double-wavelength Shimadzu spectrodensitometer scanning at 570 and 395 nm for the sample and the reference, respectively.

Mg^{2+}-Induced Aggregation. The turbidity (absorbance at 600 nm) of 1 mL of 0.5 mg of protein/mL 0.03 M phosphate buffer, pH 7.0, solution of soluble fractions of native or modified isolates produced by succesive 10–20 μL additions of 0.4 M $MgCl_2$ solution was measured. Turbidity values were taken as an index of Mg^{2+} induced aggregation.

Water-Imbibing Capacity (WIC). WIC values of soy protein isolates and their soluble and insoluble fractions were determined using a modification of the Baumann apparatus (Torgensen and Toledo, 1977). This apparatus consists of a funnel connected to a horizontal capillary. A 50 mg sample was dusted on top of the funnel filled with water. The apparatus was kept at 20 °C. The uptake of water by the sample at equilibrium was read in the graduated capillary and expressed as milliliters of water imbibed per gram of sample.

Apparent Viscosity (η_{app}). Apparent viscosities of soluble and insoluble fractions of modified isolates were measured in 8% w/w aqueous dispersions. Measurements were carried out at 20 °C in a Haake Rotavisco RV2 viscometer using an NV sensor system and a rotor speed varying from 0 to 128 rpm in 2 min and kept for 1 min at maximal speed. The apparent viscosity at 128 rpm was calculated as $\eta_{app} = GS/n$ (mPa·s), where G is an instrument factor (for the NV system, $G = 329$ mPa·s/scale grade·min), S is the scale value, and n is the rotor speed (rpm).

Gelling Capacity. Aqueous dispersions (9–11% w/w) of soluble fractions of native and modified isolates were heated at 80 °C for 30 min and then cooled overnight at 4 °C. The formation of self-supporting gel was examined by visual observation.

Table 1. Denaturation Degree and Yield of Total Isolates and Soluble and Insoluble Fractions[a]

	total isolate		soluble fraction		insoluble fraction	
soy isolate	ΔH (J/g)	D (%)	ΔH_S (J/g)	yield (% w/w)	ΔH_I (J/g)	yield (% w/w)
native	17.6	0	17.6	98.3	17.1	1.7
treated at						
80 °C/5%	4.6	74.0	5.2	95.0	1.7	5.0
80 °C/8%	5.6	68.3	7.5	55.7	3.2	44.3
80 °C/11%	6.6	62.4	9.4	29.6	5.4	70.4
80 °C/13%	7.9	54.8	11.7	27.2	6.1	72.8
80 °C/15%	9.2	47.6	14.6	27.2	7.1	72.8
treated at						
100 °C/5%	0	100	0	80.0	0	20.0
100 °C/8%	0	100	0	14.4	0	85.6
100 °C/11%	0	100	0	6.9	0	93.1
100 °C/13%	0	100	0	5.6	0	94.4
100 °C/15%	0	100	0	5.6	0	94.4

[a] ΔH, ΔH_S, and ΔH_I are the values of heat denaturation enthalpy for total isolates and soluble and insoluble fractions, respectively. $D\% = (\Delta H$ treated isolate/ΔH native isolate) × 100 is the percentage of denaturation of treated with respect to native isolate.

Foaming Properties. Foam formation was measured using a graduated glass column having a fritted glass disk (G4 type) at the bottom. N_2 gas was sparged at a flow rate (f_r) of 180 mL/min through 30.6 mL of 0.5–2 mg/mL of total or soluble fractions of native or modified isolates in 0.5 M NaCl, until a fixed volume of foam was reached ($V_f = 275$ mL); the time to reach this volume (t_f) was registered. Determinations were performed in duplicate. The volume of the remaining aqueous phase at different times until the end of bubbling was recorded. Volumes of liquid incorporated to the foam were calculated, being V_{max} (maximum volume in mL) the volume at the end of bubbling. The rate of liquid incorporation to the foam (v_i, mL/min) was determined. Foaming capacity (FC) was measured as

$$FC = V_f\,(\text{mL}) \div f_r\,(\text{mL/min}) \times t_f\,(\text{min})$$

The time for draining half the liquid incorporated in the foam at the end of the bubbling period ($t_{1/2}$ in min) was determined. Foam stability was measured as the specific rate constant of drainage, $K = 1/V_{max}t_{1/2}$ (mL^{-1} min^{-1}) (Elizalde et al., 1990).

RESULTS AND DISCUSSION

Structural Properties. The native soybean isolate prepared in our laboratory had a high yield of soluble fraction (98% in water at pH 7) (Table 1). The thermal treatment applied to aqueous dispersions of this isolate involved a heating for 30 min at 80 or 100 °C and a subsequent cooling for overnight at 4 °C. The procedure led to formation of either a viscous aqueous dispersion (at 5% isolate concentration) or a gel (at concentrations equal to or greater than 8%), a fact that was expected on the basis of previous studies (Catsimpoolas and Meyer, 1970; Hermansson, 1986). As described under Materials and Methods, total isolate and its soluble and insoluble fractions were obtained. Their properties will depend on whether the gel was formed or not, and on the gel type, both factors being dependent on the degree of denaturation and extent of protein aggregation reached.

Percentages of both soluble and insoluble fractions obtained at different heating conditions are shown in Table 1. It can be seen that the percentages are functions of two treatment variables: temperature and isolate concentration. With regard to the latter, we saw at both temperatures that the percentage of the in-

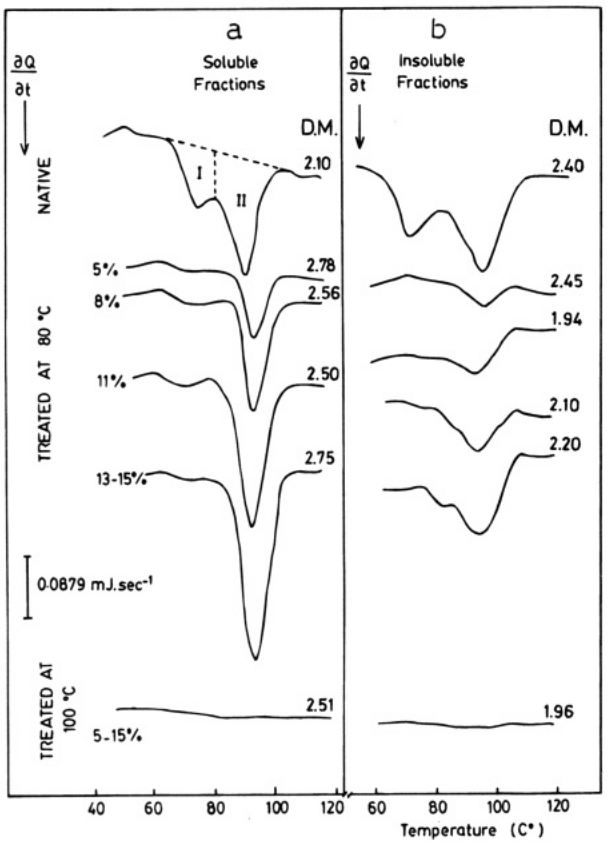

Figure 1. Differential scanning calorimetry (DSC) thermograms of 20% (w/w) aqueous dispersions of soluble and insoluble fractions of native and treated isolates. Heating rate was 10 °C/min. Peaks I and II correspond to thermal denaturation of 7S and 11S proteins, respectively. D.M.; dry matter weight (mg).

soluble fraction increases with the concentration. At concentrations equal to or greater than 8% and as a consequence of the marked increase of protein-protein interaction (gel formation), there was a sharp increase of the insoluble amount formed. At the same isolate concentration, the percentage of insoluble fraction was greater at 100 °C than it was at 80 °C, as a result of the temperature effect on the denaturation rate and protein aggregation degree (Hermanson, 1986).

Table 1 shows enthalpies of denaturation (ΔH, J/g) and denaturation percentages (D, %) of proteins present in native and heated isolates. ΔH of the native isolate was 17.6 J/g, which corresponds to $D = 0\%$, whereas those of isolates heated at 80 °C equaled D of 74.0% and 47.6% at isolate concentrations of 5% and 15%, respectively. These results indicate that, at 80 °C, denaturation was partial and that there was a protective effect among proteins which became more evident at higher isolate concentrations. All isolates treated at 100 °C showed zero enthalpy values, so they were totally denatured ($D = 100\%$).

DSC thermograms of both soluble and insoluble fractions are shown in Figure 1, parts a and b, respectively. The corresponding ΔH values are shown in Table 1. Soluble and insoluble fractions of the native isolate had two endothermic transitions (peaks I and II), with $T_{max} = 74$ and 83 °C, which can be attributed to the thermal denaturation of 7S and 11S proteins, respectively (Hermansson, 1986). Table 1 shows that ΔH values of both soluble and insoluble fractions of the native isolate were similar to the ΔH values of the total native isolate. This result indicates that 7S and 11S

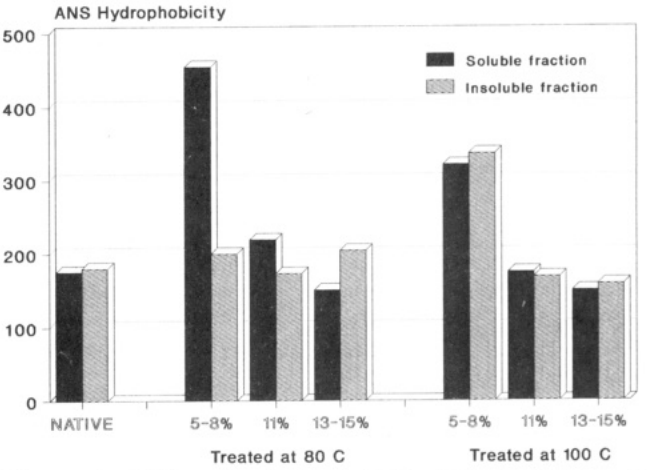

Figure 2. ANS-surface hydrophobicity (S_0) of soluble and insoluble fractions of native isolate and isolates treated for 30 min at 80 °C/5−15% and at 100 °C/5−15%.

proteins were in native state and equally distributed in both fractions, though in a different aggregation state. On the other hand, Figure 1 shows that DSC thermograms of soluble and insoluble fractions of isolates treated at 80 °C were very different from one another. In all soluble fractions obtained at 80 °C, the denaturation of 7S protein was almost total (disappearance of peak I) (Figure 1a). In the 80 °C/5% soluble fraction, the area of peak II was about 40% of the same area in the thermogram of the soluble fraction of the native isolate (60% denaturation of 11S protein). As the concentration increases (from 8% to 15%), an enrichment of the soluble fraction in native 11S protein was observed (enlarged peak II area). On the other hand, from Figure 1b, it can be deduced that insoluble fractions (80 °C/ 5−15%) were composed of proteins in a much greater denaturation state than that of corresponding soluble fractions, which suggests different distributions of native and denatured proteins between the two fractions. As the isolate concentration increases, the areas of endotherms of insoluble fractions increase, which implies a more noticeable coprecipitation of native proteins with the denatured ones. At 15% isolate concentration, peak I (7S) begins to be evident. Figure 1 also shows that in the soluble and insoluble fractions of isolates treated at 100 °C, the 7S and 11S proteins were totally denatured (so the characteristic endothermic peaks did not appear).

As a result of thermal denaturation, there were changes in the surface hydrophobicity (S_0) of proteins in both soluble and insoluble fractions of the isolate (Figure 2). S_0 values of both soluble and insoluble fractions of the native isolate were low and of the same order. This indicates, on the one hand, that the hydrophobic zones of 7S and 11S proteins present in both fractions were oriented to the interior of the molecule, which is in agreement with the typical globular structure of the native protein and, on the other hand, that hydrophobic interactions did not play an important role in the aggregation of proteins present in the insoluble fraction of the native isolate. These results agree with those obtained in DSC studies (Table 1; Figure 1). In the soluble and insoluble fractions of the treated isolates, it can be seen that S_0 was a function of treatment conditions: temperature and isolate concentration. In the soluble fraction resulting from thermal treatment at 80 °C the S_0 value was high only at low isolate concentrations (5% and 8%), conditions in

which partially denatured proteins had low tendency to aggregate, exposing their hydrophobic zones to the aqueous medium. The low S_0 of the insoluble fraction obtained in the same treatment (80 °C, 5% and 8%) suggested that the denaturation of proteins induce hydrophobic interactions in the protein aggregation process leading to the insoluble fraction. We have already seen that at 100 °C the denaturation of 7S and 11S was complete (Figure 1), so that the surface hydrophobicity of the soluble fraction in the 5% and 8% treatment should have been greater than that of the treatment at 80 °C. However, the result was a lower S_0 value in the soluble fraction and an increase of S_0 value in the corresponding insoluble fraction (Figure 2). This could be explained on the basis of the following: (a) a stronger tendency toward protein aggregation as a result of hydrophobic interactions, high temperatures, and the high degree of denaturation reached; (b) in the formation of the insoluble fraction at lower isolate concentration (5% and 8%), not all of the exposed hydrophobic zones caused by denaturation would be involved (low number of interaction points among polypeptide chains); and (c) at greater isolate concentrations (11%, 13%, and 15% at both temperatures), S_0 values of both fractions were markedly decreased during heating, because of the high degree of aggregation reached (high number of hydrophobic zones involved in interactions among polypeptide chains).

Previous work reported that in the soluble fraction of thermally denatured soy isolates there is a decrease of the basic 11S polypeptide content (highly hydrophobic) and of the β-7S subunit content, as a result of the formation of insoluble aggregates (Utsumi et al., 1984). The acidic (A) polypeptides of 11S protein have been separated in four fractions (AS I, II, III, and IV). AS IV is linked to its B-polypeptide counterpart only through noncovalent interactions, which could break due to heat treatment. The formation of soluble aggregates may be preceded by the release of AS IV; subsequently, the polymerization proceeded (Nakamura et al., 1984). AS IV polypeptide is not an integral and essential component of soy isolate gel (Utsumi and Kinsella, 1985). Therefore, it may be assumed that in soluble fractions an enrichment of AS IV occurs. Earlier work performed in our laboratory showed that this selective β–B aggregation process is clearly enhanced at high denaturation degrees (Arrese et al., 1991). In the present work, such results were confirmed by SDS–PAGE studies on soluble fractions (Figure 3). When soluble fractions of thermally treated isolates at high concentration (Figure $3b_2,c_2$) are compared with native ones (Figure 3a), we observed decreases of not only the B-11S polypeptide and β-7S subunit content but also of α- and α′-7S subunits. The same figure shows that this led to enrichment in A-11S polypeptides (peak A may be mainly composed by AS IV fraction) and other protein species (possibly whey proteins such as the Kunitz trypsin inhibitor (peak K) and agglutinin (peak Ag) according to their molecular weight (Iwabuchi and Yamauchi, 1987). In isolates treated at 80 °C/13% and 15%, the soluble fraction was enriched in A-polypeptide (partially denatured, see Figure 1a) and in whey proteins (evaluated by peak K), to contents no greater than 3.5 and 5.5 times those of the native isolate, respectively (Figure 4). On the other hand, in the soluble fraction of isolates treated at 100 °C/11%, 13%, and 15%, this tendency is more enhanced. Totally denatured A-polypeptide (Figure 1a) increases 3.3–5.0 times and

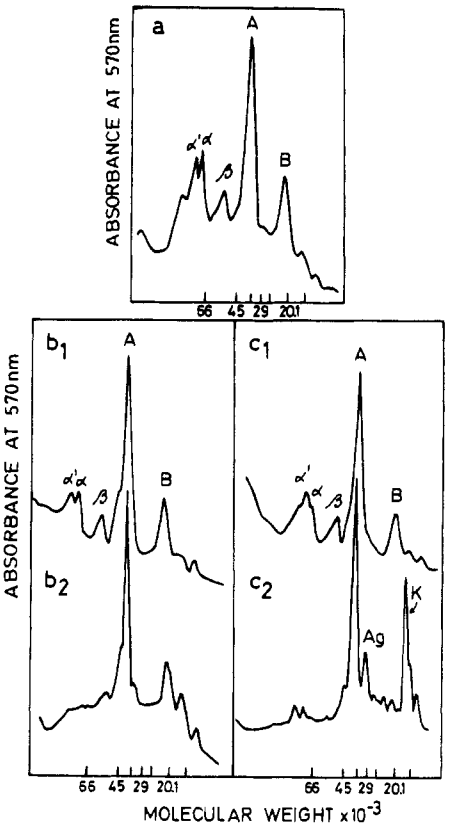

Figure 3. Densitometric scans of the electrophoretic patterns (SDS–PAGE) in the presence of 2-mercaptoethanol (5% v/v) of soluble fractions of (a) native isolate and isolates treated for 30 min at (b_1 and b_2) 80 °C/5% and 15%; (c_1 and c_2) 100 °C/5% and 15%, respectively.

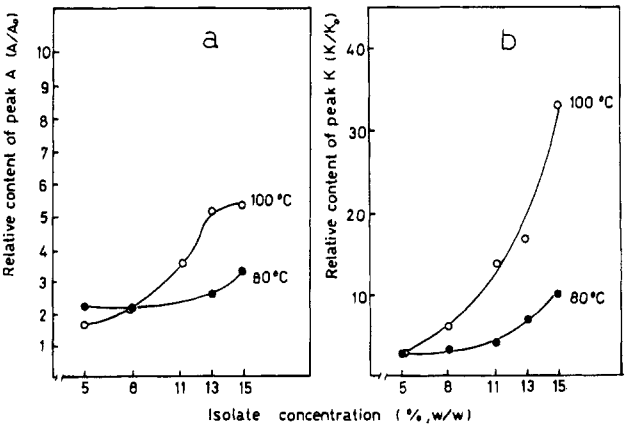

Figure 4. Variation in electrophoretic patterns from Figure 3 as a function of isolate concentration in thermal treatment at 80 and 100 °C. Variation was calculated by the ratio of the length of (a) peak A (A-11S polypeptide) and (b) peak K (Kunitz trypsin inhibitor) in the treated isolate with respect to native ones (A_0, K_0).

peak K to 14–33 times as much as the respective contents of the native isolate.

These proteins (A-11S and whey proteins) present a lower tendency to aggregation so they remain soluble even at high temperatures and concentrations.

As a consequence of thermal denaturation, the soluble fractions were also modified in their Mg^{2+}-induced aggregation capacity. This aggregation is related to the accessibility of polar groups of 11S protein which are able to form saline bridges with the Mg^{2+} and to induce aggregation (Appu Rao and Narasinga Rao, 1975). Figure 5a shows that the turbidity ($A_{600\ nm}$) of soluble fractions increased with Mg^{2+} ion concentration up to

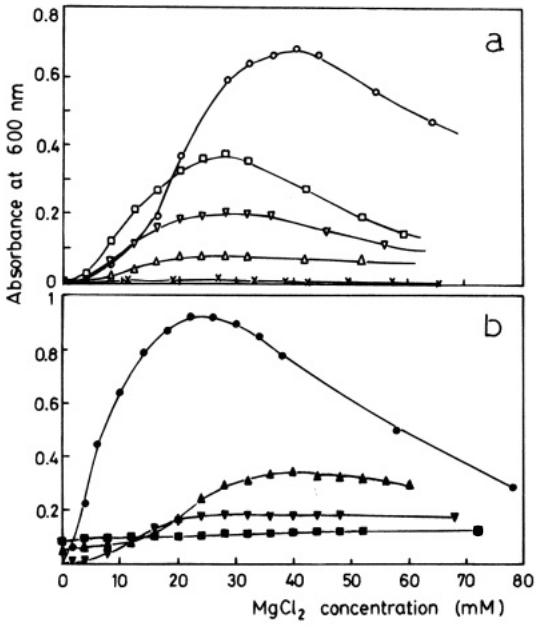

Figure 5. Turbidity (A_{600}) of soluble fraction of native isolate and isolates treated for 30 min at 80 and 100 °C, as a function of $MgCl_2$ concentration. (a) (○) Native isolate; (△, ▽, and □) isolates treated at 80 °C/ 8%, 11%, and 13%, respectively; (×) isolates treated at 100 °C/8−13%. (b) Native (●) 11S and (▲) 7S protein; (▼) 11S and (■) 7S treated at 100 °C for 30 min.

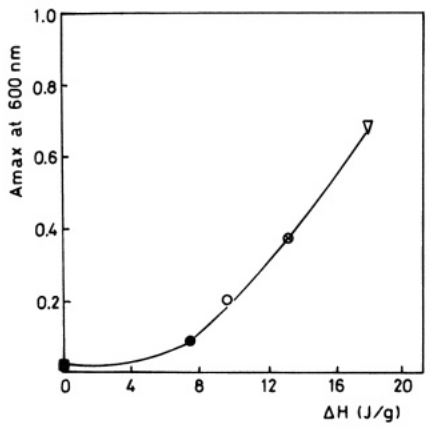

Figure 6. Turbidity maximun ($A_{600\ max}$) of soluble fractions induced by Mg^{2+} as a function of the denaturation enthalpy (ΔH). (▽) native isolate; (●, ○, and ⊗) isolates treated at 80 °C/8%, 11%, and 13%, respectively; (■) isolates treated at 100 °C/8−13%.

a maximum value. The soluble fraction of the native isolate reached the turbidity maximum ($A_{600\ max}$ = 0.683) at a concentration of 40 mM $MgCl_2$, after which the turbidity decreased, thus indicating dissociation of protein aggregates. In the soluble fractions of heated isolates, there was a decrease of both $A_{600\ max}$ and the $MgCl_2$ concentration in which the maximum was reached. This change could be attributed to the different denaturation states of 7S and 11S proteins, and this inference is made on the basis of a good correlation between $A_{600\ max}$ and ΔH values of soluble fractions (Figure 6). Studies on Mg^{2+}-induced aggregation performed with crude 11S and with a fraction enriched in 7S (70% 7S and 30% 11S), in both native and thermally denatured states (Figure 5b), have permitted us to deduce the following: (1) The aggregation capacity was mainly determined by ion Mg^{2+}−11S protein interactions. (2) The induced agregation capacity of totally denatured 11S protein was decreased but not nullified. (3) The turbidity maximum of the 11S protein was modified by the presence of 7S protein.

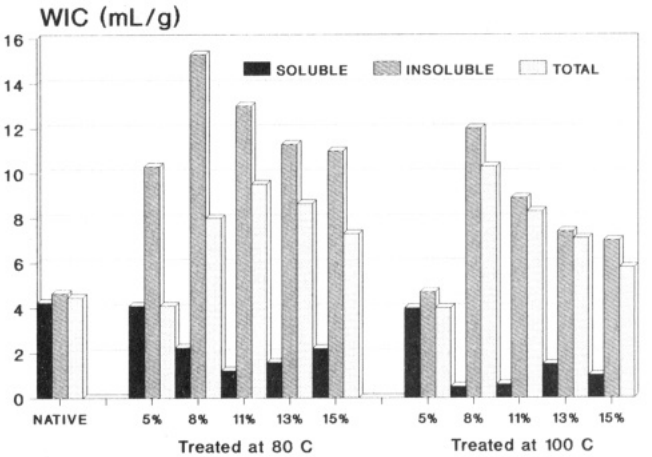

Figure 7. Water-imbibing capacity (WIC) of total isolate and soluble and insoluble fractions of native isolate and isolates treated at 80 °C/5%, 8%, 11%, and 15% and 100 °C/5%, 8%, 11%, and 15%.

If Figure 5a is now analyzed on the basis of such results, we can reach the conclusion that, for the soluble fraction of the native isolate, the turbidity maximum was at 40 mM $MgCl_2$, owing to the influence of native 7S on the 11S−Mg^{2+} interaction. However, in soluble fractions from isolates heated at 80 °C, the decrease of the turbidity maximum at 28 mM $MgCl_2$ can be caused by the fact that the 7S protein is denatured (Figure 1). In soluble fraction of isolates treated at 100 °C and at high isolate concentration, one would expect that the result would be the same as that of denatured 7S and 11S (Figure 5b). The low turbidity values obtained in the presence of Mg^{2+} would be explained by the disappearence of some species caused by insolubilization.

All of the information provided by results from DSC, hydrophobicity, PAGE and Mg^{2+}-induced aggregation studies on various soluble and insoluble fractions obtained from native and thermally treated isolates at several concentrations and temperature conditions has allowed us to deduce that, apart from having different yields, they differ markedly in their structural properties and in their composition. We then proceed with the analysis of how these structural and composition changes affect the functional properties of soluble and insoluble fraction and, therefore, how they affect those of the total isolate.

Functional Properties. We have seen that the native soy isolate obtained in our laboratory had a high water solubility and that the thermal treatment brought about a loss of solubility which, depending on heating conditions, led to variable amounts of soluble and insoluble fractions (Table 1). Such fractions, in turn, possessed very distinct functional properties as described in previous work performed on commercial soy isolates (Sorgentini et al., 1991). In this regard, Figure 7 shows the water-imbibing capacity (WIC) of soluble and insoluble fractions of native and thermally treated isolates. The graph shows that the insoluble fraction obtained from thermally treated isolates had high WIC, a feature not found in the soluble fraction. At both 80 and 100 °C, the insoluble fraction with the highest WIC was achieved in treatments at 8% isolate concentration. Below this level, there was no gelling during heating and, therefore, no network able to absorb and to retain water. Causes under which treatments performed at concentrations equal to or greater than 11% led to

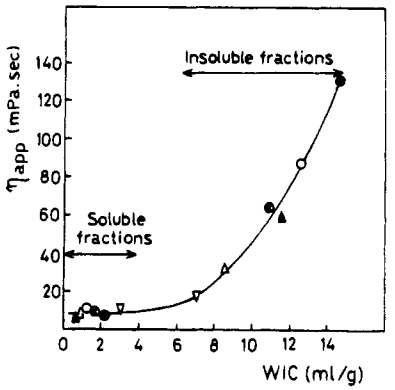

Figure 8. Apparent viscosity (η_{app}) of aqueous dispersions (8% w/w) as a function of WIC of soluble and insoluble fractions of isolates treated at 80 °C/(●) 8%, (○) 11%, and (⊗) 13% and at 100 °C/(▲) 8%, (△) 11%, and (▽) 13%.

insoluble fractions with lower WIC than at 8% isolate concentration differed according to heating temperatures. At 80 °C, the insoluble fractions obtained at concentrations equal to or greater than 11% showed decreased WIC because they contained increasing amounts of native 11S (Figure 1), which, in turn, had a low water-imbibing capacity. At 100 °C, increasing contents of B-11S and 7S subunits (Figures 3 and 4), which had high aggregation capacity, induced the formation of very compact aggregates, thus reducing the water-imbibing capacity of the insoluble fraction.

Even though the concentration leading to maximum WIC in the insoluble fraction was 8% at 80 °C, the maximum WIC of the total isolate occurred at 11% (Figure 7), a concentration value in which the proportion of the insoluble part was greater than 65% (Table 1). Among all isolates, that having the highest WIC was prepared at 100 °C/8%, a condition in which both WIC and the insoluble percentage were high (WIC = 12.0%, insoluble fraction = 85.6%). These results were due to the fact that the water-imbibing capacity of the isolate is a consequence of both the WIC of the soluble and insoluble fractions and the percentage of these fractions in the isolate.

Previous work has shown that the rheological behavior of aqueous dispersions of commercial and laboratory soy isolates is a function of their imbibed water ratio, which depends on the WIC (Yao et al., 1988). Similar results were obtained here on soluble and insoluble fractions of laboratory isolates. Figure 8 shows that soluble fractions (low WIC values) contributed a little to the viscosity, whereas the insoluble fractions, with greater WIC, possess a high viscosity.

While the water-imbibing capacity depends on the insoluble fraction of isolates, other properties such as the capacity to form gels and foams depend mainly on the soluble fraction. A comparative test on the gelling capacity of soluble fractions in native and thermally treated isolates was performed. Results show that at a concentration of 9% w/w, the gelling capacity was preserved only in soluble fractions obtained from native isolates and from those thermally treated at 80 °C/5–8%. In the other soluble fractions, this capacity was gradually lost; this was reflected either by the necessity of using greater concentration (11% w/w) to form the gel (samples at 80 °C/11–15% and 100 °C/5%) or by a total loss of the gel formation capacity (samples 100 °C/8–15%). The increase of isolate concentration and temperature of the thermal treatment applied to the isolate would lead to a decrease, in the soluble fraction,

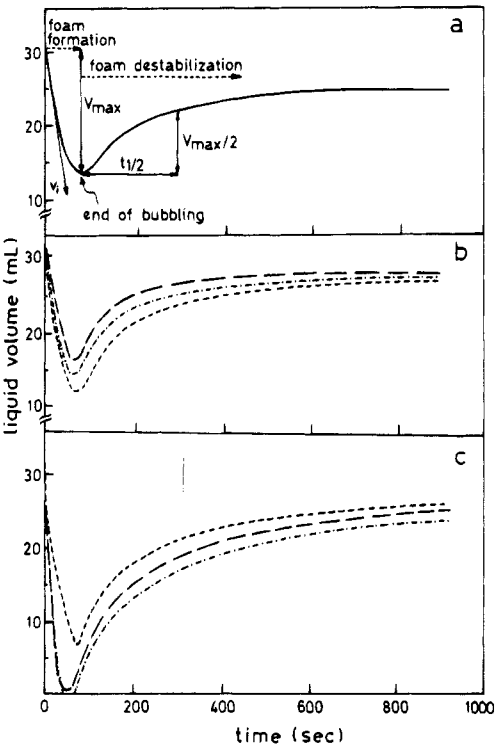

Figure 9. Remanent liquid volume as a function of time during N_2 bubbling (liquid incorporation into the foam corresponding to foam formation) and after it (liquid drainage corresponding to foam destabilization). Foaming assays were performed with aqueous dispersions (0.5 mg/mL) of soluble fractions of (a) (—) native isolate and treated isolates at (b) 80 °C and (c) 100 °C: (- - -) 5%, (— · —) 11%, and (— —) 15%.

of those particular protein species B-11S and α, α′, β-7S with essential contribution in the aggregation process (Figures 3 and 4). Therefore, these proteins are responsible for gel formation.

Figure 9 shows profiles obtained by determination of the foaming properties of different soluble fractions. According to Yu and Damodaran (1991), slight variations of the ionic strength in the 0–0.1 M range led to major changes in the foam stability. For this reason, we measured the foam capacity in 0.5 M NaCl solutions to avoid possible ionic strength variations caused by differences in the saline content of soluble fractions. For native isolate (Figure 9a), we were able to see that the first part of the curve corresponds to the foam formation (incorporation of liquid into the foam), whereas the second part corresponds to foam stability once the bubbling ceased (loss of liquid from the foam, or liquid drainage). In the same figure, the physical meanings of v_i, V_{max}, and $t_{1/2}$ parameters are also indicated. In foaming profiles obtained with soluble fractions of thermally treated isolates, we observed modifications in the curve region related to foam formation (v_i and V_{max} variations, Figure 9b,c). In soluble fractions of isolates treated at 80 °C/8–15% and at 100 °C/5–15%, the v_i values (incorporation rate of liquid into the foam) were higher than those of the native isolate (Figure 10). This indicates that the greater the isolate concentration and temperature of the thermal treatment, the greater the tensioactivity of the resultant soluble fractions. This may be the result of an increase, in the soluble fraction, of protein species with smaller molecular size and greater flexibility, as a consequence of the following simultaneous processes that occur along the course of the thermal treatment: (i) dissociation of 7S and 11S oligomeric structures (Yamauchi et al., 1991); (ii) gradual

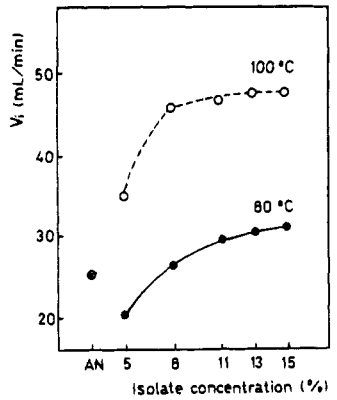

Figure 10. Rate of liquid incorporation into the foam (v_i) as a function of isolate concentration in thermal treatment. N, native isolate. All foaming assays were performed with aqueous dispersions of soluble fractions at 0.5 mg/mL.

Table 2. Foaming Parameters of Soluble Fractions of Native and Treated Isolates

soluble fraction	V_{max} (mL)	$t_{1/2}$ (min)	K (1/min·mL)
native isolate	17.7	3.4	0.019
isolate heated at			
80 °C/5%	20.7	2.6	0.019
80 °C/8%	17.7	1.7	0.033
80 °C/11%	17.0	2.1	0.028
80 °C/13%	17.0	2.3	0.026
80 °C/15%	16.4	2.3	0.026
100 °C/5%	24.4	2.5	0.016
100 °C/8%	30.6	1.9	0.017
100 °C/11%	30.6	3.2	0.010
100 °C/13%	30.6	3.0	0.011
100 °C/15%	30.6	2.8	0.012

denaturation of 7S, 11S, and whey proteins (Figure 1; Table 1); and (iii) selective enrichment in A-11S polypeptide and whey proteins (Figures 3 and 4).

Table 2 presents values of V_{max}, $t_{1/2}$, and resultant K (specific rate constant of drainage, which is a measure of foam stability) (Elizalde et al., 1990) for soluble fractions of native and heated isolates. We only saw an important increase of foam stability (lower K values) in soluble fractions of isolates obtained at 100 °C and at higher protein concentrations (equal to or greater than 11%). In fact, these are the particular fractions that, besides showing proteins in a totally denatured state, also present an important increase in A-11S polypeptide and in whey proteins (Table 1; Figure 4a,b).

Results presented until now correspond to foaming tests performed on protein dispersions at a concentration of 0.5 mg/mL. To study the effect of an increase of protein concentration in the foamed solution on the foam forming and stabilization processes, we carried out tests at greater concentrations (1 and 2 mg/mL). As samples, we used the soluble fraction of isolates in the native state and also those of isolates treated at 5%/80 °C and at 5%/100 °C. For all soluble fractions tested, the foaming capacity (FC) values varied in the 0.87–0.91 range, which means a high and equivalent retention of N_2 during the foam formation by bubbling (Figure 11a). The concentration increase from 0.5 to 2 mg/mL led to a significant increase of v_i and to a decrease in K of thermally treated samples (Figure 11b,c). For the sample in native state, the same protein concentration increase led to modifications of less importance in both parameters. Such results indicate, in the first place, that it is possible both to incorporate and to stabilize more liquid into the foam by increasing the protein concentration of the foamed solution and, in the second place, that the effect of this concentration increase is greater in denatured isolates than in the native one, so that differences owing to structural modifications and protein composition become more evident.

An additional variable must be considered in studies on foaming properties of total isolates: the isolate solubility. Accordingly, we selected isolates which differed not only in the type of protein species and denaturation degree but also in the soluble fraction percentage (native isolate, 80 °C/15% and 100 °C/15%, with solubilities of 98%, 25%, and 5%, respectively). In Figure 11d–f values of FC, v_i, and K for aqueous dispersions of the above-mentioned isolates at 0.5, 1, and 2 mg/mL are observed. Figure 11d shows for isolate dispersions of low concentration (0.5 mg/mL) that the FC was lowered while the isolate solubility decreased. When concentration increased up to 2 mg/mL, FC reached a value of 0.90, both in native isolates and in heated ones, which indicates an efficient retention of N_2 during bubbling, equivalent to that of the soluble fractions (Figure 11a). The v_i of heated isolates increased with concentration in such a way that, at 2 mg/mL, it surpassed the corresponding value of the native isolate (Figure 11e). The low v_i obtained with the 100 °C/15% isolate at 0.5 mg/mL was caused by its low solubility. However, with an increase in the concentration, this particular isolate yields the maximum v_i since it contains the soluble fractions having the best foaming properties (Table 2). On the other hand, Figure 11f shows that foams made from 80 °C/15% and 100 °C/15% isolate dispersions yield high K values (very low stability). In foams obtained from dispersions at increasing concentration of such isolates, this constant sharply decreased to foam stabilities similar to those of native isolates. Results from Figure 11d–f considered as a whole verify that the foam forming and stabilizing properties were determined both by the characteristics of the soluble fraction and by the concentration of this fraction in the foamed solution. What remains to be determined is the influence of the insoluble fraction in the foam forming and stabilization processes. To this end, we performed tests using a 0.5 mg/mL dispersion of native isolate to which we added 0.5 mg/mL of all insoluble fractions obtained from isolates modified by several different thermal treatments. Comparisons with V_i, K, V_{max}, and $t_{1/2}$ foaming parameters of the native isolate (Table 2; Figure 11e) permitted us to determine that the foam formation phase was not modified (average $v_i = 26.2 \pm 2.4$ mg/mL), since the K value increased up to an average value of 0.026 ± 0.003, which represents a decrease in the foam stability. This is mainly caused by the reduction of $t_{1/2}$ (average $t_{1/2} = 2.3 \pm 0.2$ min) since V_{max} values remain almost constant (average $V_{max} = 17.3 \pm 0.9$ mL). This foam stability loss is explained through interferences caused by insoluble particles in the formation of the protein lamella (Walstra, 1989).

Conclusions. Protein isolates in both native form (without thermal treatment) and thermally modified form (under different conditions of temperature and isolate concentrations) allowed us to prepare a wide range of soluble and insoluble fractions. These fractions differed markedly one to the other in yield, composition, and structural characteristics of their constitutive proteins. Such differences can be ascribed to the fact that the major proteins present in the isolates (glycinin, 11S,

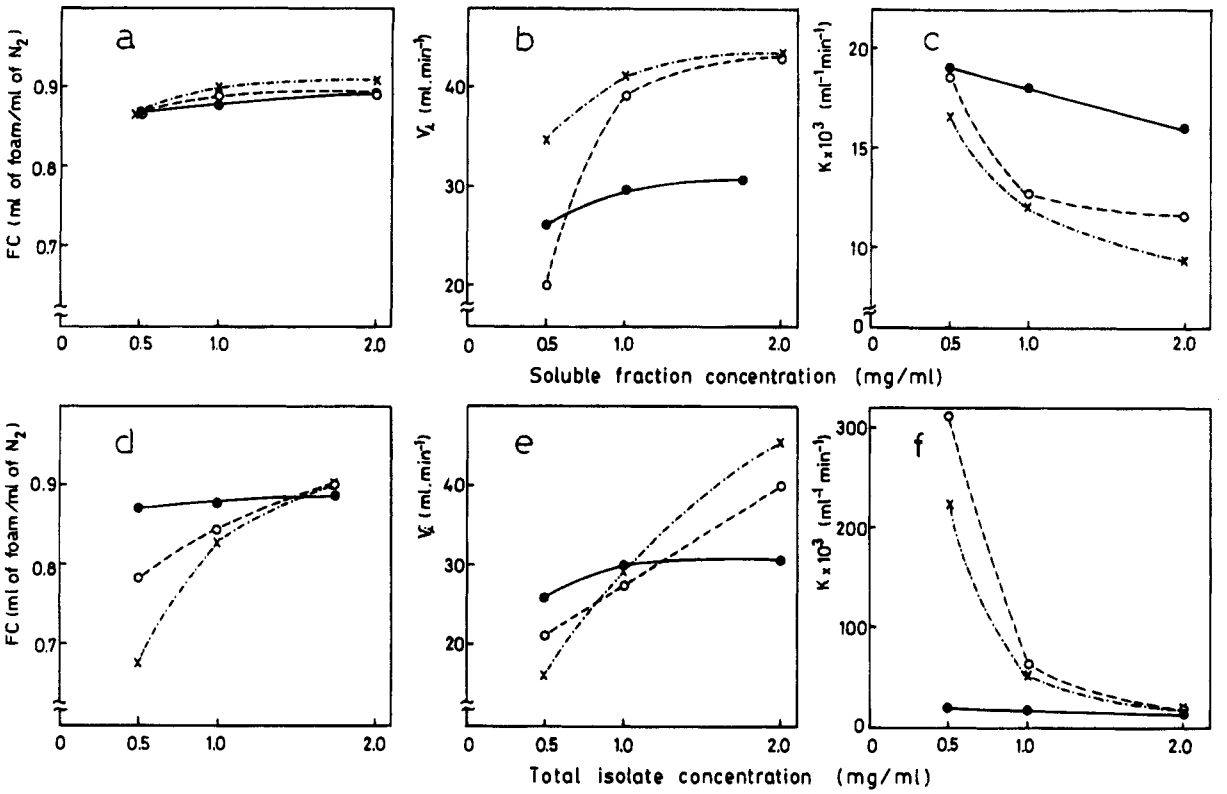

Figure 11. Foaming capacity (FC), rate of liquid incorporation into the foam (v_i), and specific rate constant of drainage (K) as a function of sample concentration in the foaming assay. (a–c) Soluble fraction of (●) native, (○) 80 °C/5%, (×) 100 °C/5%, respectively. (d–f) Total isolate (●) native, (○) 80 °C/15%, and (×) 100 °C/15%, respectively.

and β-conglycinin, 7S) were differently affected by the several thermal treatment applied to the isolates.

In this regard, the treatment at 80 °C, which produced a total denaturation of 7S protein but only a partial denaturation of 11S protein, allowed us to obtain, for increasing isolate concentration, soluble and insoluble fractions progressively enriched in native 11S protein. In the treatment at 100 °C, denaturation of 11S protein was total, because of which soluble and insoluble fractions did not differ in the denaturation degree but in the type of protein species present (a phenomenon also observed at 80 °C though in a lesser extent). The greater the isolate concentration in the treatment, the lesser was the content of B-11S polypeptide and of α-, α′- and β-7S subunits in the soluble fraction, which resulted in an enrichment in A-11S polypeptide and whey proteins. These differences were reflected by surface hydrophobicity values and by the tendency to Mg^{2+}-induced aggregation.

The characteristics of composition and structure of soluble and insoluble fractions had a marked influence on their functional properties and, consequently, on those of the total isolate. The water-imbibing capacity (WIC) and the viscosity of the insoluble fraction increased with the degree of protein denaturation, and both were a function of the type of protein species involved in the aggregation process. When this process was sufficiently advanced, it led to loss of gelation capacity since this property depends on the possibility of new protein–protein interactions in the soluble fraction. This fraction, in turn, determined the surface properties of the total isolate. The enrichment of the soluble fraction in proteins with lower molecular weight and greater flexibility (whey proteins and A-11S polypeptide) led to a marked increase of the capacity to form and to stabilize foams. Finally, by controlling temperature and protein concentration in the thermal treatment, the functionality of the isolate can be modulated, thus enhancing properties of the soluble fractions at the expense of those of the insoluble ones (or vice versa). Another possibility presented with this procedure is the obtention of multipurpose isolates that conserve, in partial form, the functionality of both fractions.

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