Relationships between Different Hydration Properties of Commercial and Laboratory Soybean Isolates

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Functional properties related to water protein interactions of soy protein isolates depend on the structural and aggregation characteristics of their major components (storage globulins 7S and 11S) that could be modified by the preparation procedure, thermal and/or chemical treatments, and drying methods. Commercial and laboratory isolates with different functionalities resulting from their structural modifications were compared. Isolates with high solubility or excessive thermally induced insolubilization or compact calcium-induced aggregates caused low water-imbibing capacity (WIC) values. The highest WIC results from the balance between intermediate solubility and the formation of aggregates with good hydration properties. The apparent viscosity of dispersions of commercial (spray dried) and laboratory (lyophilized) isolates depends on the WIC, the morphology and size of the particles, and the interaction of the hydrated particles. The hydration properties and viscosity of protein isolate suspensions were strongly determined by the amount and properties of the insoluble fraction.

Keywords: *Soybean protein; isolates; hydration properties; water-imbibing capacity; viscosity*

INTRODUCTION

Intentional protein modifications have been widely used to improve functional properties of soy protein isolates (SPI) in order to increase their use as ingredients in the food industry. SPI can be used in foodstuffs as water-binding agents or to increase viscosity and to form gels or foams (*1*). Changes in structural and functional properties may be achieved by thermal or chemical treatments (*2*−*5*).

Some of the most important functional properties of soy isolates are related to their interaction with water. The degree of denaturation and/or aggregation of proteins during the preparation of the isolates is an important factor that affects functional properties such as solubility, water absorption, and viscosity (*1*, *6*). Solubility generally decreases with increasing surface hydrophobicity. However, there are some other factors that may affect protein solubility, such as structural effects, thus showing the limitation of the "hydrophobicity equals insolubility" argument (*7*, *8*).

Water-imbibing capacity (WIC) could be defined as the amount of water that is spontaneously imbibed by soy proteins and that is physically held within a protein matrix (*9*). This parameter is of great importance with regard to the quality of several food products. Previous results obtained in our laboratory with commercial SPI have shown that the insoluble fraction of isolates is the most important factor in the WIC of the isolate (*4*). Fully denatured proteins have a higher tendency to aggregate at higher surface hydrophobicity (*10*). As a consequence, denatured 11S and 7S globulins (the major components of the SPI) are the main components of the insoluble fraction. Both the degree of aggregation and the aggregate size have an influence on the WIC. In addition, the presence of salts, principally from divalent cations, modifies the properties of soy protein aggregates.

The viscosity and rheological properties of protein dispersions are highly sensitive to the moisture content [expressed as total water to imbibed water ratio (T/I), determined by the WIC] and protein concentration. The improvement of hydration properties obtained by thermal treatments of soy isolates allows more viscous dispersions to be obtained at lower protein concentration (*11*).

In the present work we compared some functional properties related to water−protein interactions of commercial isolates (with quite different properties due to different processing conditions) and laboratory isolates, which have been modified by thermal, acid, or thermal−acidic treatments (at different protein concentration, temperature, pH, and time conditions, with or without calcium). Such properties have been evaluated in previous studies in our laboratory in terms of solubility, WIC, T/I ratio, and viscosity (*4*, *6*, *10*−*12*).

The objective of this work was to study the relationships between the WIC, solubility, and apparent viscosity of commercial and laboratory soy isolates and to propose an explanation in terms of the characteristics of the modified proteins.

MATERIALS AND METHODS

Defatted soy flour was provided by Sanbra S.A. and commercial soy isolates by Sanbra S.A., Protein Technologies International, and Societe Industrielle des Oleagineux.

Native Soy Isolates (N). Defatted soy flour was extracted for 2 h at 20 °C with alkalinized water, pH 8.0 (flour/water ratio = 1:10) and centrifuged at 8500*g* for 30 min at 20 °C.

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The supernatant was adjusted to pH 4.5, stored at 4 °C for 2 h, and centrifuged again at 8500*g* for 20 min at 5 °C (standard procedure). The resulting precipitate was dissolved in water, adjusted to pH 8.0, and freeze-dried. Native isolates had a denaturation enthalpy (ΔH) of 17.0 ± 0.5 J/g.

Modified Soy Isolates. All modified laboratory isolates were prepared according to the standard procedure followed by different treatments: TH (thermal treatment at high protein concentration: 80−100 °C, 5−100 min, 8−13% $w_{isolate}/w_{solution}$, pH 7, ΔH = 0−8 J/g); TL (thermal treatment at low protein concentration: 80−100 °C, 30−360 min, 3−5% w/w, pH 7, ΔH = 0−8 J/g); TLCa (thermal treatment at low protein concentration with the addition of $CaCl_2$: 90 °C, 100 min, 3% w/w, 0.5−32.1 mg of Ca/g of protein, ΔH = 0 J/g); THCa [addition of different amounts of $Ca(OH)_2$ to the isoelectric precipitate with high protein concentration (≈43% w/w), neutralized with NaOH and then thermally treated at 90 °C for 100 min; final Ca concentration = 2.6−28.8 mg/g protein, ΔH = 0 J/g]; TLA (thermal−acid treatment at low protein concentration: 90 °C, 30 min, 6% w/w, pH 1.6, ΔH = 0 J/g); TLAp (same as TLA at pH 1.6 with precipitation at pH 4.5 after acid treatment); LA (acid treatment at low protein concentration: 20 °C, 60 min, 6% w/w, pH 1.0−3.5, ΔH = 3.8−12.5 J/g); LAp (same as LA at pH 1.6 and precipitation at pH 4.5 after acid treatment). All modified isolates were freeze-dried.

Water Solubility. Isolates were dispersed in water at 1% w/v by stirring for 1 h at 30 °C and centrifuged at 8500*g* for 30 min at 15 °C. Soluble proteins in the supernatant were determined by using the biuret method (*13*), and solubility was expressed as percentage of total protein. Determinations were performed in triplicate.

Differential Scanning Calorimetry (DSC). Samples (∼14 mg) of 20% aqueous dispersions were placed in sealed aluminum pans and analyzed at 10 °C/min from 20 to 120 °C (DSC Polymer, Rheometric Scientific). An empty double pan was used as a reference. The equipment was calibrated at the same heating rate by using indium, lauric acid, and stearic acid (p.a.) as standards. Thermal denaturation enthalpies (ΔH in joules per gram of dry matter) were calculated from the endothermic curves. Determinations were performed at least in duplicate.

WIC. WIC was determined using a modification of the Baumann apparatus (*14*). It was expressed as milliliters of water imbibed per gram of sample. The ratio of total to imbibed water (T/I) was calculated (*15*). Determinations were performed at least in duplicate.

Apparent Viscosity. η_{app} (in centipoise) was measured in isolate aqueous dispersions (1.6−16% w/w) at 20 °C in a Haake Rotavisco RV2 viscosimeter. A Sensor system NV was employed, and a rotor speed was varied from 0 to 128 rpm in 2 min and kept for a minute at maximum speed. The apparent viscosity at 128 rpm was calculated as $\eta_{app} = GS/n$ (cP), where G is an instrument factor (cP/scale grade min), S is the scale value, and n is the rotor speed (rpm). Determinations were performed at least in duplicate.

Particle Density. Particle density was determined by pycnometry using xylene (*16*). Xylene can separate the particles comprising the isolate without penetrating into their pores. The weight of the displaced xylene was determined and the equivalent volume was measured on the basis of the density of the particles comprising the isolate. The mean particle density was calculated as $\rho_A = P_A/V_A$, where P_A is the weight of the isolate (100 ± 30 mg of isolate) and V_A is the volume of the particles in the isolate (equivalent to the volume of xylene displaced by the isolate). Determinations were performed by duplicate.

Scanning Electron Microscopy (SEM). SEM assays were conducted on 1% w/v dispersions of commercial and laboratory soy isolates in xylene. Samples were placed in a bronze stub and coated with gold, the specimens being observed with a Philips SEM 505 at an acceleration voltage of 25 kV. Determinations were performed at lest in quintuplicate.

Statistical Analysis. Data were analyzed by analysis of variance and significance of differences between means by the Fisher's test (Systat version 5.0). An α level of 0.05 was used to determine significance.

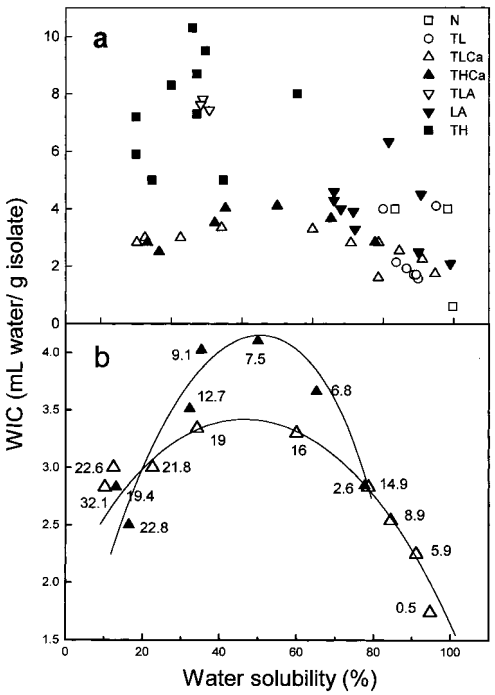

Figure 1. (a) Relationship between WIC and water solubility of laboratory soy isolates. Nomenclature of samples is detailed under Materials and Methods. **(b)** Relationship between WIC and water solubility for samples with calcium (TLCa and THCa) in expanded scale. Numbers correspond to the amount of calcium expressed as milligrams of Ca per gram of protein. Each point represents a mean value of replicates.

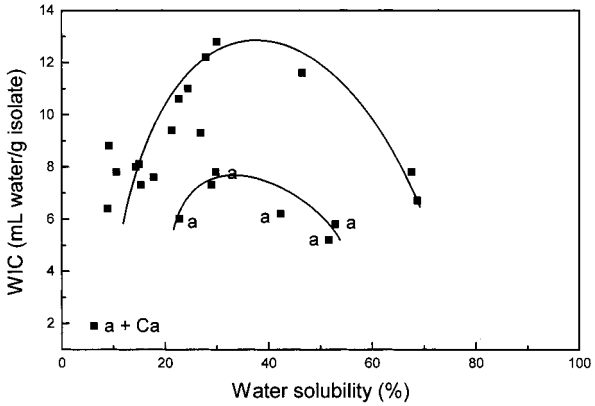

Figure 2. Relationship between WIC and water solubility of commercial soy isolates. Isolates with enthalpy of denaturation of (■) 0−2.7 J/g or (■a) >7 J/g. (**■a + Ca)** same as ■a with the addition of 10.4 mg of Ca/g of protein (see Materials and Methods). Each point represents a mean value of replicates.

RESULTS AND DISCUSSION

Relationship between Solubility and WIC. Figures 1 and 2 depict the WIC as a function of solubility in water of the isolates that were prepared in the laboratory and commercial isolates, respectively. As reported previously (*6*), isolates of intermediate solubility (20−60%) showed the highest WIC in each group ($p < 0.05$). Isolates N, constituted mainly by native 7S and 11S globulins, showed a high %S but low WIC. The same results were observed with isolates TL, composed of the same proteins (totally or partially denatured by heat treatment at low protein concentration). Denatur-

ation under those conditions did not cause a loss in solubility due to the diminution of the aggregation (*8*). TLCa samples were obtained via the same thermal treatment but in the presence of $CaCl_2$. When increasing quantities of $Ca(OH)_2$ were added to the isoelectric precipitate dispersed in a minimum amount of water (≈43% protein) and then thermally treated, the resulting isolates (THCa**)** showed behavior similar to that of TLCa. In Figure 1b a decrease of %S can be observed with the increasing concentration $CaCl_2$ in samples THCa and TLCa. When intermediate solubility values were reached, only a slight increase ($p < 0.05$) in WIC was observed for both samples (maximum WIC ≈ 3.2 and 4.0 mL/g to TLCa and THCa, respectively), suggesting that the presence of $CaCl_2$ induced the formation of hydrated insoluble aggregates. The compact nature of these protein aggregates may be the reason they were unable to absorb more water. In the same figure it is possible to observe that during thermal treatment, a high content of Ca (>22 mg/100 g of protein) is necessary to reach <20% solubility. This treatment led to the formation of insoluble and compact isolates, which were consequently difficult to hydrate. Isolates with >70% solubility had a low proportion of insoluble proteins, which are responsible for the WIC (*4*). Consequently, the preparation of isolates with high WIC would require a balance between the degree of insolubilization and the nature of the aggregates. On the other hand, the isolates obtained by thermal treatment at high protein concentration but without the addition of Ca, TH, displayed <60% solubility and intermediate to high values of WIC (maximum = 12.1 mL of water/g of isolate) (Figure 1a). In contrast to the isolates heated in the presence of Ca, TH isolates would be composed of insoluble aggregates with a good water absorption capacity.

The results related with the acid-treated isolates are also presented in Figure 1a. Isolates LA, in which the component proteins were dissociated and partially denatured (partial denaturation of 7S and total denaturation of 11S) (*5*, *17*), showed >60% solubility and a low WIC (<7 mL/g). Isolates obtained by thermal treatment in acid media, TLA, in which 7S and 11S fractions were totally denatured ($\Delta H = 0$ J/g) and which had a certain degree of deamidation and hydrolysis (*18*), showed a slightly lower %S than the corresponding LA but a higher WIC. When isolates LA and TLA were obtained from long-stored flours (>6 months), they showed the same degree of denaturation but a high WIC. This result has been previously reported (*5*). Isolates having a high proportion of the insoluble fraction were obtained by elimination of salts and soluble peptides (which do not contribute to the WIC) from isolates LA and TLA **(**LA_p and TLA_p**)** by precipitation in their p*I*. These isolates had consequently higher WIC (>16 mL/g, data not shown).

With regard to the commercial isolates (Figure 2) that are generally submitted to thermal treatments at high protein concentration and to spray-drying processes, they showed the same WIC and %S values as the TH isolates.

The decrease of enthalpy value of DSC transitions reflects the degree of protein denaturation due to previous isolates treatments and/or a greater tendency of protein aggregation during the DSC scan. The latter could be ascribed to an increase in surface hydrophobicity of the proteins as result of structural modifications. Nevertheless, the contribution of this last effect to the total enthalpy value is small in comparison to that of the denaturation effect (*19*). On the basis of this consideration, it is possible to estimate the denaturation degree of different commercial isolates. According to enthalpy values of DSC transitions, two well-defined populations of isolates were observed: those with $\Delta H > 7$ J/g (a) and the rest with $\Delta H = 0-2.7$ J/g (Figure 2). At the same solubility, the latter (formed by partially or totally denatured proteins) showed a 2-fold increase in WIC values in comparison to the less-denatured isolates (a) ($p < 0.05$). In both groups, the highest values of WIC were obtained at intermediate solubility (30−40%) ($p < 0.05$). Usually, commercial isolates contained low Ca concentrations (0.2−2.4 mg/g of isolate). Instead, the isolate a **(**Ca**)** in Figure 2 corresponding to a calcium proteinate (prepared by the addition of 10.4 mg of Ca/g during neutralization at high protein concentration) showed very low WIC and %S.

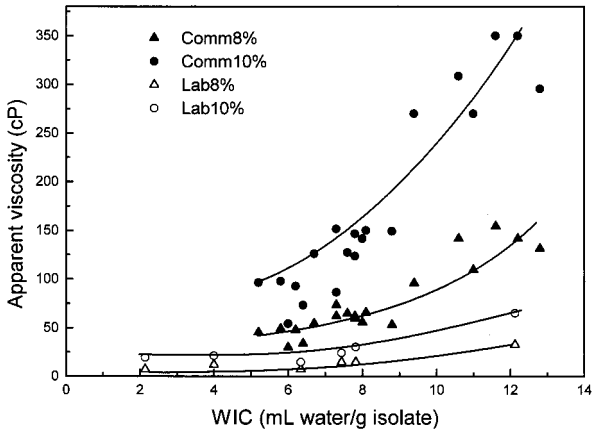

Figure 3. Apparent viscosity of commercial (Comm) and laboratory (Lab) isolates as a function of WIC. Measurements were performed in isolate aqueous dispersions (8 and 10% w/w) at 20 °C. Each point represents a mean value of replicates.

Relationship between WIC and Apparent Viscosity. As reported previously, the rheological properties as well as the viscosity of aqueous soy protein dispersions are related to the hydration capacities of their composing proteins (*6*, *11*). Figure 3 shows that the apparent viscosity (η_{app}) of commercial and laboratory aqueous dispersions is a function of the WIC. In commercial isolates the increase of η_{app} with WIC was more evident at higher protein concentrations (10%). For the same WIC range, laboratory isolates (Lab) showed values of η_{app} up to 10 times lower than the commercial isolates ($p < 0.05$). In the same concentration range (8−10%), laboratory isolates that were treated in acid media produced dispersions of lower viscosity (<25 cP) even at WIC values >12 mL/g. These results may be attributed to the presence of highly soluble and partially hydrolyzed and deamidated−dissociated proteins (data not shown) (*18*). According other workers (*20*, *21*) not only hydrolysis but also deamidation decreases the formation of network-like structures by increasing the surface charge and decreasing the molecular mass.

For all laboratory isolates, a concentration >10% was necessary to increase over 100 cP the apparent viscosity of the dispersions. These results suggest that the apparent viscosity depends on the interaction between soluble and insoluble proteins with water and between the hydrated particles. The latter are affected by the morphology of the protein particles, especially from the

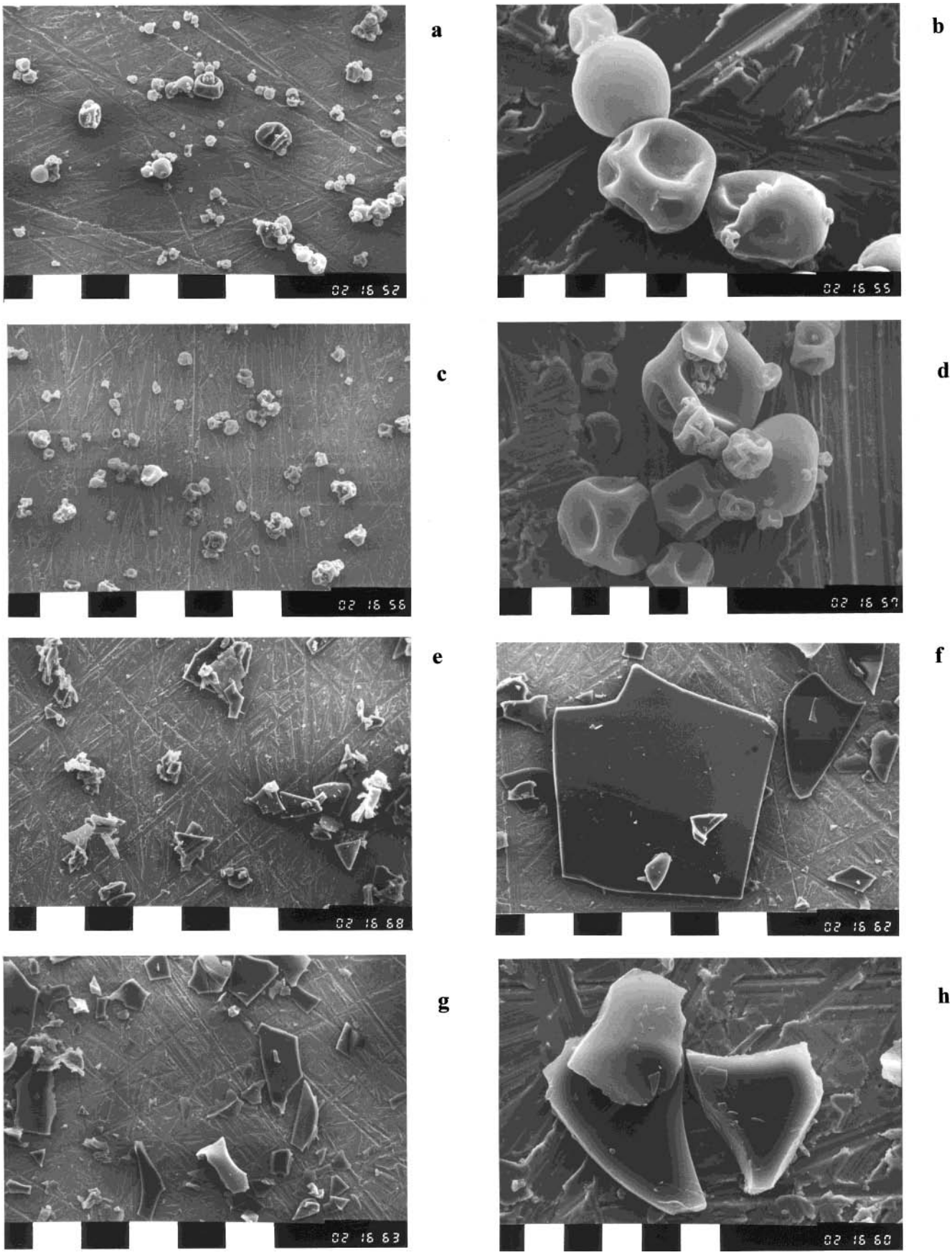

Figure 4. SEM of soy protein isolates: (a−d) commercial (Sanbra, Brazil); (e, f) native (N); (g, h) thermally treated (TL) laboratory isolates. Magnifications: (a, c, e, and g) 150×; (b, d, f, and h) 1200×. Horizontal bars represent 100 and 10 μm for 150× and 1200× magnifications, respectively. (Figure is reproduced here at 67% of the original size.)

insoluble fraction. By optical microscopy and density measurements, differences in the size and morphology of particles were observed between commercial and laboratory isolates. Small spherical and porous particles

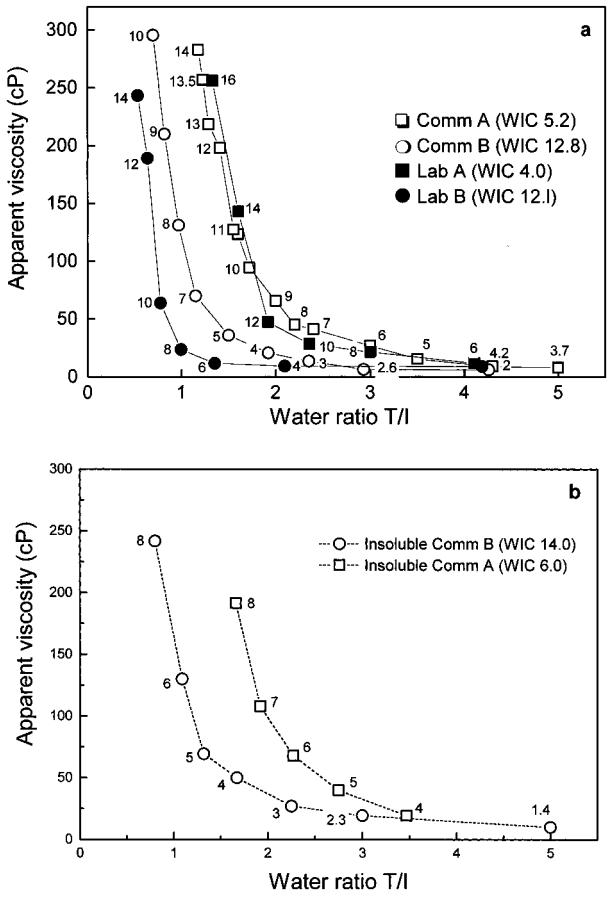

Figure 5. Apparent viscosity as a function of total water/imbibed water ratio (T/I) of **(**a**)** commercial (Comm) and laboratory (Lab) isolates with low and high WIC and **(b)** insoluble fractions (with low and high WIC) of commercial isolates. Each point represents a mean value of replicates.

(particle density of ~1 g/cm^3) were observed in the former (spray dried), and in the latter isolates (lyophilized) irregular, flat, and compact plaques were seen (particle density ≥ 1.2 g/cm^3) (Figure 4). It would be expected that the interaction between particles of commercial isolates would be higher than those between laboratory ones, due the higher number of more hydrated particles per gram of isolate.

Different values of η_{app} could be obtained by varying the concentration of the isolate in the dispersion in a range of 2−16%, due to a modification of the T/I ratio (Figure 5a). As was previously reported (*15*), for a single isolate, η_{app} increases when T/I tends to 1 (all water is absorbed). When commercial and laboratory isolates were compared at the same WIC, the resulting curves were coincident for only those isolates with low WIC (~4−5 mL/g), in which high values of apparent viscosity (250−300 cP) were reached at concentrations of 14−16% with a T/I = 1.5. Different curves were obtained even at the same water absorption values (WIC_{Comm} = 12.8 and WIC_{Lab} = 12.1) for isolates with high hydration capacity. Results obtained with the insoluble fractions of commercial isolates A and B with low and high WIC, respectively, are plotted in Figure 5b. Those curves from each isolate and from their corresponding insoluble fraction were almost the same, suggesting that the viscosity of dispersions of protein isolates was strongly determined by the amount and properties of the insoluble particles. From the above results, it is possible to infer that high viscosity could be reached with isolates

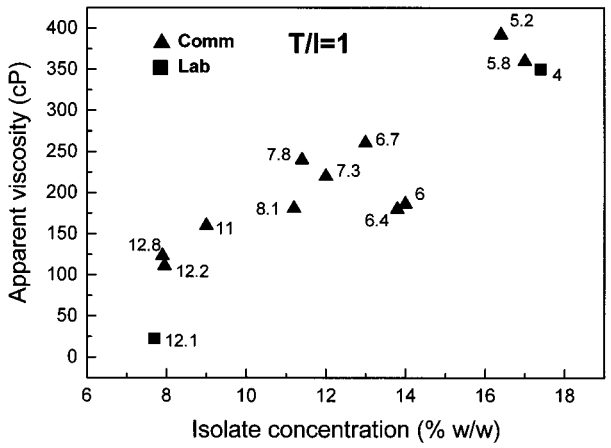

Figure 6. Relationship between apparent viscosity and isolate concentration of aqueous isolates dispersions with a ratio of T/I = 1. Numbers correspond to WIC values of isolates. Each point represents a mean value of replicates.

with low WIC (group A) and that the viscosity strongly increased with the concentration of the isolate. It is important to point out that the number of hydrated particles was also increased together with the particle−particle interactions. Under those conditions, a high viscosity was obtained, although part of the water was not imbibed by the particles (T/I > 1).

With regard to the rheological behavior of commercial dispersions at high concentrations (with η_{app} > 200 cP), a pseudoplastic behavior was observed with an important tixotropy, suggesting that a high interaction between particles at the beginning decreased as a function of time (*11*). The orientation of the aggregates in the direction of the flux or their partial rupture increased the particle mobility. The same behavior was observed with the laboratory isolates with low WIC (Lab A, data not shown). For isolates with high WIC, dispersions with η_{app} > 200 cP were obtained at lower concentrations (9% of Comm B, >12% of Lab B) (Figure 5a). In this case, the number of hydrated particles was lower, but because they do have a high hydrodynamic volume, they absorbed all of the available water. The high viscosity would consequently be determined by the interaction between these hydrodynamic volumes (which may overlap at T/I < 1). The rheological behavior was slightly tixotropic for the Comm B dispersions and slightly reopectic for the Lab B dispersions (data not shown). The difference could be explained on the basis that although both dispersions form stable structures, the latter were still able to absorb water during the measurement. The difference between commercial and laboratory isolates with high WIC is based on the difference in the morphology of the hydrated particles that depends on the drying procedure. This behavior could be extrapolated to the other tested isolates. Figure 6 shows the difference in viscosity of commercial dispersions that had absorbed all available water (T/I = 1), suggesting that the parameter T/I was not the only factor affecting the viscosity of a single isolate. As the WIC increased, lower concentrations were needed to absorb all of the water, suggesting a weak interaction between highly hydrated particles. Two laboratory isolates with extreme WIC values were also include in Figure 6 showing the same behavior.

Different treatments that can be used in the processing of certain isolates could affect the hydration capacity of the soluble fraction. To visualize this effect, apparent

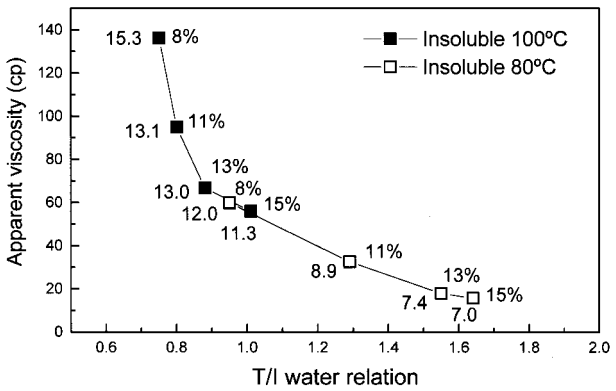

Figure 7. Apparent viscosity as a function of total water/imbibed water ratio (T/I) of insoluble fractions of laboratory thermally treated isolates (80−100 °C, 30 min, 8−15% protein concentration). WIC values of insoluble fractions are indicated at the left of each point. Each point represents a mean value of replicates.

viscosity was plotted as a function of T/I for the insoluble fractions of laboratory isolates with thermal treatments at different conditions (8−15% protein concentration, 30 min at 80−100 °C) (Figure 7). The different soluble fractions did not influence the viscosity at the assayed concentrations (8%) because η_{app} was <20 cP (data not shown). At 8% water, dispersions of the insoluble fractions allowed increasing apparent viscosity as a function of their WIC to be obtained. At either 80 or 100 °C the highest WIC was obtained with the insoluble fraction of isolates treated at lower concentration (8%). However, it is important to point out that the WIC of the insoluble fraction increased with the solubility of the isolate (solubility of isolated heated to 80 °C at 15% protein, changed from 27.2 to 55.7%; solubility of the isolates treated at 100 °C and at 13 to 8% protein changed from 5.6 to 14.4%) (*4*).

CONCLUSIONS

Laboratory and commercial isolates of intermediate solubility showed the highest WIC. High solubility or excessive insolubilization induced by heating or highly compact calcium-induced aggregates caused low WIC values. The degree of denaturation of the constituent proteins also influences the value of WIC. Thus, to obtain isolates with high WIC, a balance between the degree of insolubilization−denaturation and adequate characteristics of the resulting aggregates would be required.

The apparent viscosity (η_{app}) of aqueous dispersions of commercial and laboratory isolates increased with WIC, the latter having 10 times lower values. The viscosity decreased even more in acid-treated isolates, with partially hydrolyzed and deamidated−dissociated proteins. The apparent viscosity seemed to depend on the interaction between soluble and insoluble proteins with water and on the morphology, size, and interaction of the hydrated particles.

The WIC and the ability to form viscous dispersions of protein isolates were strongly determined by the amount and properties of the insoluble fraction. The conditions originating suspensions of good hydration properties would be those yielding isolates with intermediate values of both WIC of the insoluble fraction and solubility.

This systematic study on the functional properties related to protein−water interactions will be useful to estimate the characteristics of soy protein isolate that largely will determine its potential utilization and best applications as an ingredient in formulated foods, epecially to evaluate the effect of processing variables on viscosity, WIC, and solubility and to select the better performance for a specific food. As examples, an isolate with reduced solubility between 30 and 60% by thermal treatment, without calcium addition, will lead to an excellent additive to be used as a water-binding agent; and the addition of low calcium content and heating at low protein concentration allowed isolates with high solubility that could be used as ingredient in nutritive beverages to be obtained.

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