Relation between Solubility and Surface Hydrophobicity as an Indicator of Modifications during Preparation Processes of Commercial and Laboratory-Prepared Soy Protein Isolates

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Because water solubility is the main hydration property of proteins, solubility values of commercial and laboratory soy protein isolates, prepared under different conditions, were comparatively analyzed. In contrast, the surface hydrophobicity manifested by proteins is a physicochemical property that determines, to a great extent, the tendency of protein molecules to aggregate and so to lose solubility. On these grounds, the solubility of isolates was analyzed as a function of the surface hydrophobicity of their proteins, and, as a result, three well-defined groups of laboratory isolates were identified: (A) native, (B) partially or totally denatured with high solubility and surface hydrophobicity, and (C) totally denatured with low solubility and surface hydrophobicity. Commercial isolates could not be included in any of these groups; they were grouped as (A′) partially native and (C′) totally denatured. Solubility values in these two groups were similar to those of group C, but the surface hydrophobicity levels were much lower. The different processes leading to the groups mentioned above are discussed, along with the way the soy proteins are influenced by the specific preparation conditions, namely, protein concentration, chemical or thermal treatments, presence of salts, drying, and phospholipid addition, among others.

Keywords: *Soy proteins; soy isolates; solubility; surface hydrophobicity; modified isolates*

INTRODUCTION

The functional properties of soy isolates, associated with hydrodynamic behavior as much as with protein−protein interactions, reflect the composition and structure of their major components, 11S (glycinin) and 7S (β-conglycinin) globulins, and depend basically on the degree of dissociation, denaturation, and/or aggregation of those proteins (Kinsella, 1979; Kinsella et al., 1985; Utsumi et al., 1984).

A number of papers have been published on processing treatments that may change the protein structure and modified specific functional properties (Ohren, 1981; Wu and Inglet, 1984; Yamauchi et al., 1991; Rhee, 1994). The structural modifications may be accomplished by thermal or chemical treatments, the former one being the more frequently used (Hermansson, 1978; Kinsella, 1979; Yamauchi et al., 1991). Mild acid treatment is an interesting alternative that has also been used on soy protein isolates (Matsudomi et al., 1985; N Guyen Thi Quynh et al., 1992) or on 11S soybean protein (Wagner and Gueguen, 1995). Almost all acid treatments included heating, which added other modifications such as hydrolysis and deamidation. The effect of different treatments depends on the different conditions (temperature, time, protein concentration, pH, ionic strength).

Many results have been obtained since work began with soy protein isolates in our laboratory, with the aim of determining the structural−functional relationships. Studies have started with commercial soy isolates (Wagner and Añón, 1990; Arrese et al., 1991; Sorgentini et al., 1991; Wagner et al., 1992) and continued with those prepared in the laboratory in native or denatured (totally or partially) states and with variable aggregation degree (Sorgentini et al., 1995; Wagner et al., 1996; Petruccelli and Añón, 1994). By means of this research, proper preparation conditions were established to encompass a wide range of functional properties.

In the present study, we have used the results obtained with commercial soy isolates, which have variable solubilities and denaturation degrees, and with soy isolates prepared in our laboratory in native state and modified by applying different thermal or chemical treatments. In summary, the samples employed here covered a wide range of modification that translates into important changes at the structural or functional level. The highly soluble native isolate, called "native" because every type of protein denaturation, either chemical or physical, was avoided during its preparation, has been taken as reference soy isolate.

Previous results demonstrate the importance of surface hydrophobicity on hydrodynamic properties, such as solubility, of soy, milk, and meat proteins (Li-Chan et al., 1984; Hayakawa and Nakai, 1985; Nakai et al., 1986).

The objective of this work was to establish general trends or relations between the solubility and surface hydrophobicity values exhibited by the commercial isolates and isolates prepared in our laboratory, in terms of their conditions of preparation.

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Table 1. Preparation Conditions for Native Soy Isolates Prepared in the Laboratory

	standard preparation procedure							
isolate	extraction (pH−*t*−F/W)[a]	first centrifn (rpm−*t*−*T*)[b]	precipn pH	second centrifn (rpm−*t*−*T*)	washing in distilled water	redissolution pH	initial concn for freeze-drying (%)	ΔH^c (J/g of dry matter)
1	8−1 h-1/20	10000−20 min-5 °C	4.5	5000−10 min-5 °C	No	8	5	17.2 ± 0.5
2	8−1 h-1/20	10000−20 min-5 °C	4.5	5000−10 min-5 °C	No	8	3	17.4 ± 0.4
3	8−2 h-1/10	10000−30 min-4 °C	4.5	5000−15 min-4 °C	No	7	10	16.8 ± 0.5
4	8−2 h-1/10	5600−15 min-20 °C	4.5	6500−20 min-4 °C	Yes	8	4	17.6 ± 0.3
5	8−2 h-1/10	5600−15 min-20 °C	4.5	6500−20 min-4 °C	Yes	8	6	17.9 ± 0.4

[a] pH−*t*−F/W is pH−time−relation flour/water. [b] rpm−*t*−*T* is rpm−time−temperature. [c] ΔH values are the mean of at least two determinations.

Table 2. Treatments Inducing Protein Denaturation with Low Solubility Losses (Protein Aggregation Process Prevented) in Laboratory Soy Proteins[a]

isolate[b]	enrichment in fraction 7S or 11S (α)	$CaCl_2$ addition (mg of Ca/g of protein) (β)	thermal treatment (*P*−*T*-*t*)[c] (χ)	reducer treatment (δ)	dialysis against water 48 h−4 °C (ϵ)	base or acid treatment (*P*−pH−*t*)[d] (ϕ)	initial concn for freeze-drying	ΔH^e (J/g)
6					yes		4%	15.6 ± 0.5
6a	7S				yes		4%	12.0 ± 0.4
6b	11S				yes		4%	18.5 ± 0.5
7				yes	yes		4%	17.3 ± 0.5
7a	7S			yes	yes		4%	11.7 ± 0.3
7b	11S			yes	yes		4%	10.7 ± 0.3
8				yes + urea 6M	yes		4%	0
8a	7S			yes + urea 6M	yes		4%	0
8b	11S			yes + urea 6M	yes		4%	0
9						10%-pH 9−60 min	10%	10.0 ± 0.6
10						6%-pH 3.5−60 min	6%	12.5 ± 0.4
11						6%-pH 2−60 min	6%	8.1 ± 0.2
12						6%-pH 1−60 min	6%	6.3 ± 0.2
13			5%−40 °C−30 min				5%	17.1 ± 0.6
14			5%−60 °C−30 min				5%	15.0 ± 0.5
15			5%−77 °C−30 min				5%	8.4 ± 0.3
16			5%−100 °C−30 min				5%	0
17			3%−90 °C−20 min				3%	n.d.
18			3%−90 °C−45 min				3%	0
19			3%−90 °C−100 min				3%	0
20			3%−90 °C−240 min				3%	0
21			3%−90 °C−360 min				3%	0
22			5%−80 °C−30 min				4%	4.6 ± 0.1
23			5%−100 °C−30 min				4%	0
24		0.5	3%−90 °C−100 min				3%	0
25		5.9	3%−90 °C−100 min				3%	0
26		8.9	3%−90 °C−100 min				3%	0
27		14.9	3%−90 °C−100 min				3%	0

[a] Standard preparation procedure was made as in native isolates (Table 1) according to the following: isolates 6, 6a, 6b, 7, 7a, 7b, 8, 8a, 8b, and 9, same as in isolate 3; isolates 13−21 and 24−27, same as in isolate 1; isolates 22, 23, and 10−12, same as in isolate 4. Treatment α was made after the first centrifugation of standard preparation procedure (Table 1). Treatment δ was performed with 0.05% sodium sulfite−pH 7 at 4% protein concentration and room temperature during 3 h. Treatments β, χ, δ, and ϵ were performed after the standard preparation procedure. Treatment ϕ was made after the second centrifugation of standard preparation procedure for isolates 9, and after washing for isolates 10−12. [b] Isolates with subindex a and b were obtained, respectively, by differential precipitation at pH 6.4 and 4.8, successively, instead of isoelectric precipitation. [c] *P*−*T*-*t* is protein concentration-temperature-time. [d] *P*-pH-*t* is protein concentration-pH value-time. [e] ΔH values are the mean of at least two determinations. [f] nd, not determined.

MATERIALS AND METHODS

Chemical reagents of analytical grade were obtained from Merck, Mallinckrodt, and Sigma Chemical Co. Defatted, solvent-free soy flour, prepared under controlled conditions (to avoid protein denaturation), was provided by Sanbra S.A. Brazil. Commercial soy isolates used in this work were provided by Sanbra S.A., Ralston Purina (currently Protein Technologies International), and Societe Industrielle des Oleagineux. Their average composition (% w/w) was as follows: protein (N × 6.25), 86.6 ± 3.0; moisture content, 6.5 ± 1.2; ash, 3.5 ± 0.5; and lipids, 2.2 ± 0.5 (of which some 70−80% were phospholipids).

Preparation of Isolates. *Native Soy Isolates.* To prepare soy isolates with their proteins in native state (thus termed "native" isolates), procedures were followed that preserved protein structure, although they differed slightly in some of the basic steps. The defatted soy flour was extracted for 1 or 2 h at room temperature by using distilled water adjusted to pH 8.0 with 2 N NaOH (flour/water ratio of 1:10 or 1:20). The suspension was centrifuged at 5600−10000 rpm (GSA-rotor, Sorvall RC 5B refrigerated superspeed centrifuge) for 20 or 30 min at 4 or 20 °C to retain the supernatant, which, in turn, was adjusted to pH 4.5 (isoelectric point of storage soy globulins) with 1 N HCl, kept for 2 h at 4 °C, and centrifuged again for 20 min at 4−5 °C. The precipitate so obtained, either washed or not in distilled water, was redissolved in water to a protein concentration of 3−10% w/v by neutralization and pH adjustment to 7.0 or 8.0, using 0.5−2 N NaOH at room temperature. The isolate so prepared was then freeze-dried (Thermovac Industries Corp. freeze-dryer). The different combinations of conditions used to prepare the native isolates are summarized in Table 1 (isolates 1−5). All native isolates have sodium and calcium contents <13 and <1.3 mg of ion/g of isolate, respectively.

Modified Soy Isolates. Tables 2 and 3 show the treatment conditions leading to detectable changes in the protein denaturation and aggregation states. Table 2 includes the treatments that induced different denaturation degrees without

Table 3. Thermal Treatments Inducing Protein Denaturation with High Solubility Losses (by Protein Aggregation Promoted) in Laboratory Soy Isolates[a]

isolate	$CaCl_2$ addition (mg of Ca^{2+}/g of protein) (α)	thermal treatment ($P-T-t$)[b] (β)	initial concn for freeze-drying (%)	Δ*H* (J/g)
28		8%−80 °C−30 min	4	5.6 ± 0.2
29		11%−80 °C−30 min	4	6.6 ± 0.3
30		13%−80 °C−30 min	4	7.9 ± 0.3
31		8%−100 °C−30 min	4	0
32		11%−100 °C−30 min	4	0
33		13%−100 °C−30 min	4	0
34		8%−90 °C−100 min	8	0
35		13%−90 °C−100 min	13	0
36		10%−98 °C−5 min	10	6.8 ± 0.2
37		10%−98 °C−30 min	10	0
38		10%−98 °C−5 min	10	3.0 ± 0.1
39		10%−98 °C−30 min	10	0
40	19	3%−90 °C−100 min	3	0
41	21.8	3%−90 °C−100 min	3	0
42	22.6	3%−90 °C−100 min	3	0
43	32.1	3%−90 °C−100 min	3	0

[a] Standard preparation procedure was made as in native isolates (Table 1) according to the following: isolates 28−33, same as in isolate 5; isolates 34, 35, and 40−43, same as in isolate 1; isolates 36 and 37, same as in isolate 3; isolates 38 and 39, same as in isolate 9. Treatments α and β were performed after the standard preparation procedure. [b] $P-T-t$ is protein concentration-temperature−time.

causing important solubility losses in the isolates (isolates 6−27). Table 3 includes treatments done under conditions leading to important isolate solubility losses (isolates 28−43).

Water Solubility. Isolates were dispersed in water to 1% w/v under gentle and constant magnetic stirring for 1 h in a bath set at 29−30 °C. They were then centrifuged at 10000 rpm (SS34-rotor Sorvall RC 5B refrigerated superspeed centrifuge) for 30 min at 15 °C, and protein concentration was determined on the supernatant according to the biuret method (Gornall et al., 1949). Measurements were done in duplicate.

Differential Scanning Calorimetry (DSC). Samples (15−20 mg) of 20% dispersions in water were hermetically sealed in aluminum pans, using an empty double pan as reference. The samples were analyzed at 10 °C/min in a range of 20−120 °C using a DuPont model 910 calorimeter or DSC Polymer Laboratories equipment (Rheometric Scientific). The equipment was calibrated at a heating rate of 10 °C/min by using indium, lauric acid, and stearic acid (p.a.) as standards. Areas below the endothermic curves were measured to calculate the corresponding thermal denaturation enthalpies (Δ*H* in joules per gram of dry matter). All tests were repeated at least twice.

Surface Hydrophobicity (H_0). Values of H_0 were determined by the hydrophobicity fluorescence probe [with 1-anilino-8-naphthalene sulfonate (ANS)] according to the method reported by Kato and Nakai (1980). Protein dispersions (1 mg/mL) were prepared in 0.01 M phosphate buffer (pH 7.0) with or without NaCl (0.1−1 M), stirred for 2 h at 20 °C, and centrifuged at 6500 rpm for 20 min (SS34-rotor Sorvall RC 5B refrigerated superspeed centrifuge). 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 0.5 to 0.005 mg/mL; a volume of 3 mL of each diluted sample was then added with 40 mL of ANS (8.0 mM in 0.1 M pH 7.0 phosphate buffer solution). Fluorescence intensity (FI) was measured at 365 nm (excitation) and 484 nm (emission) using a Perkin-Elmer 2000 fluorescence spectrometer. The FI reading was calibrated by adjusting it to a value of 80 (1x) with 15 mL of ANS in 3 mL of methyl alcohol (Parker HPLC grade). The initial slope of the FI versus protein concentration plot (calculated by linear regression analysis) was used as an index of protein surface hydrophobicity (H_0).

RESULTS AND DISCUSSION

Relationship between Solubility and Surface Hydrophobicity. If the water solubility (%S) of laboratory-prepared isolates is plotted as a function of surface hydrophobicity (H_0), a trend is found in which the

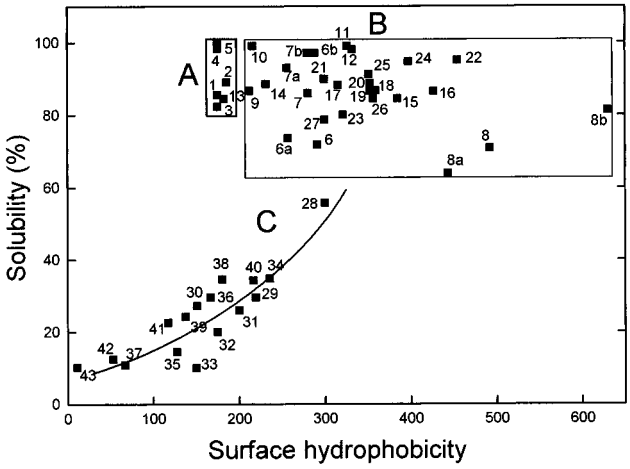

Figure 1. Laboratory soy isolates sorted as a function of the relationship between water solubility and surface hydrophobicity.

greater the H_0, the greater the solubility (Figure 1). In principle, this seems to be contradictory because proteins of high surface hydrophobicity would be expected to show low water solubility owing to their natural tendency to aggregate by hydrophobic interactions. However, bearing in mind the conditions under which the isolates were prepared, an explanation can be found for the results of the figure: first, values are not observed to follow a sole trend, suggesting that solubility is not exclusively determined by the degree of exposure of hydrophobic zones. By employing the available information on the preparation conditions of isolates and structural parameters such as the denaturation enthalpy (Tables 1−3), it was possible to sort the samples into three groups: A, B, and C. Isolates of groups A and B have similarly high water solubility ($\%S_A > 80\%$ and $\%S_B > 70\%$) but differ mainly in the denaturation state. Group A includes native laboratory isolates ($\Delta H \geq 16.8$ J/g of dry matter) with a surface hydrophobicity of 170−200 (isolates 1−5 and 13). The different conditions used in the isolate preparation stages (Table 1), namely, extraction, centrifugation, precipitation, washing, pellet redissolution, and freeze-drying, were mild enough to keep the native structure of β-conglycinin (7S) and glycinin (11S), the soybean storage globulins. DSC

thermograms of the isolates of this group are characterized, therefore, for presenting both the 7S and 11S denaturation peaks. Isolate 3 presented the lowest solubility among the native samples; it was brought to pH 7 during its redissolution stage and freeze-dried to a concentration of 10%. In turn, isolates 4 and 5 possess high solubility, suggesting that the washing of the precipitate with water (a step that contributes to eliminate salts, whey proteins, and sugars), the redissolution to pH 8, and the low protein concentration during freeze-drying have all a positive effect on the final isolate solubility. The washing step also explains the higher ΔH values of isolates 4 and 5 by enrichment of the storage soy globulin.

Group B isolates (6−27) possess H_0 values ranging from 210 to 640; this group is composed mostly of samples thermally treated under different conditions of time, temperature, and medium or else treated with denaturing chemical agents (urea, acidic pH) but always at low protein concentration (≤6%) (Table 2). Although these isolates reached different denaturation degrees, as evidenced by the H_0 values, no considerable solubility losses were found (%S > 60) because the aggregation process is minimized by the low protein and Ca concentrations (<15 mg of Ca/g of protein). In this group, isolates 9, 10, and 14 had the lowest H_0 (210−231) owing to incipient denaturation induced by pH 9 and 3.5 (isolates 9 and 10, with ΔH values of 10 and 12.5 J/g, respectively) and by gentle heating at 60 °C (isolate 14 with $\Delta H = 15.0$ J/g). Isolates 8 and 8b possess the highest surface hydrophobicity ($H_0 = 492$ and 640, respectively). These isolates and isolate 8a have been treated with 0.05% sodium sulfite as reducing agent in a 6 M urea denaturing medium. Isolates 8a and 8b were enriched in 7S and 11S globulins, respectively. In these isolates, the presence of 6 M urea causes both a total protein denaturation ($\Delta H = 0$) and a partial reduction of disulfide bonds of AB-11S subunits (Petruccelli and Añón, 1995). Although reduction of disulfide bridges caused partial liberation of peptide B-11S, insoluble aggregation did not occur to a high degree because the procedure did not involve thermal treatment. If reduction treatment was carried out at pH 8 with heating, it led to irreversible insoluble precipitation (Thanh and Shibasaki, 1976). Elimination of reducing agent and urea by dialysis after these treatments allows the aggregations of denatured polypeptides through hydrophobic interactions and disulfide bridges, with the consequent partial loss of solubility.

Among isolates of group B are some with intermediate H_0 values ($250 < H_0 < 450$), resulting from different treatments: dialysis, reduction plus dialysis, acid treatment at pH ≤ 2, or thermal treatment under different conditions of temperature and time (Table 2). Dialyzed isolates 6 (normal composition of 7S and 11S globulins), 6a (7S enriched), and 6b (11S enriched) have ΔH values similar to those of native constitutive proteins (Sorgentini and Wagner, 1999). These results agree with the idea that the dialysis process preserves the native structure of soy proteins but can induce slight dissociation−aggregation processes (Wagner and Gueguen, 1995). If a reducing treatment with 0.05% sodium sulfite is performed before dialysis, only isolate 7b (11S enriched) is partially denatured ($\Delta H = 10.7$ J/g). By comparison between 7S (6a, 7a, and 8a) and 11S (6b, 7b, and 8b) enriched isolates, it can be seen that the latter have higher surface hydrophobicity and solubility (Figure 1). The presence, in the 11S enriched isolates, of the highly hydrophobic basic B polypeptide of 11S globulin explains their high surface hydrophobicity (Thanh and Shibasaki, 1976). Otherwise, the lower solubility of 7S enriched isolates could be explained by their contamination with polymeric forms of 11S in the preparation procedure. On the other hand, isolates treated in acidic medium (pH 1−2) are partially denatured (ΔH values of 8.1 ± 0.2 and 6.3 ± 0.2 J/g for isolates 11 and 12, respectively) without solubility loss. The partial denaturation degree was due to the selective denaturation of 11S globulin, which is more sensitive to acid medium than protein 7S (Wagner et al., 1996).

Among the thermally treated isolates, isolates 16 and 22 are the most hydrophobic, and they keep a high water solubility, in contrast to isolate 8 (%S > 85). Those isolates were prepared by thermal treatment of 5% aqueous dispersions of washed native isolate (isolate 4) that causes their partial (isolate 22, $\Delta H = 4.6$ J/g) or total (isolate 16, $\Delta H = 0$ J/g) denaturation. The low protein concentration, the moderate heat treatment, and the low salt concentration (due to washing of isoelectric precipitate with water and the absence of Ca addition) are the factors that allow us, by controlling the aggregation process, to obtain highly soluble isolates (Sorgentini et al., 1995).

At high protein concentrations (≥8%), thermal treatments applied on native soy isolate dispersions induce a degree of protein aggregation such that it results in gel (Sorgentini et al., 1995). The existence of gels indicates the formation of a protein network able to absorb a high amount of water. After water is removed from the gels by freeze-drying, the protein network persists in the dehydrated isolate, leading to the formation of aggregates of lower water solubility or dispersibility. The same aggregation−insolubilization process was observed for samples treated in the presence of calcium. The presence of divalent cations may induce aggregation by ionic interactions, mainly through the glycinin (11S fraction) protein that possesses binding sites for calcium and magnesium (Appu Rao and Narasinga Rao, 1975a,b). This property allows formation of aggregates during the industrial production of milk-like beverages or tofu (soy cheese). Therefore, the degree of protein aggregation and so isolate insolubilization increases with temperature and calcium concentration during the treatment. These isolates are represented in curve C, where it is observed that the lower the solubility, the lower the surface hydrophobicity exposed by the proteins of the isolate. As the surface hydrophobicity is determined by the soluble proteins of the isolate, the result of curve C can be explained in two ways: (a) the protein species undergoing aggregation are the more hydrophobic, so that only the hydrophilic ones remain soluble; and (b) as the proteins aggregate, they hide or occlude the hydrophobic zones, leaving part of the proteins as soluble aggregates of low surface hydrophobicity. Options a and b are not mutually exclusive, and both would explain why isolates, on losing solubility, also lose surface hydrophobicity.

In light of the behavior presented by laboratory isolates, it is possible now to analyze the solubility and surface hydrophobicity values of commercial isolates.

Figure 2 indicates that commercial isolates do not behave exactly as the laboratory ones, but still two groups can be clearly identified. One, called C′ (%S and H_0 values respond like those in group C isolates),

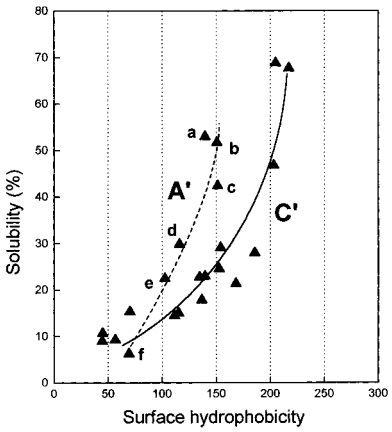

Figure 2. Commercial soy isolates sorted as a function of the relationship between solubility and surface hydrophobicity correlation.

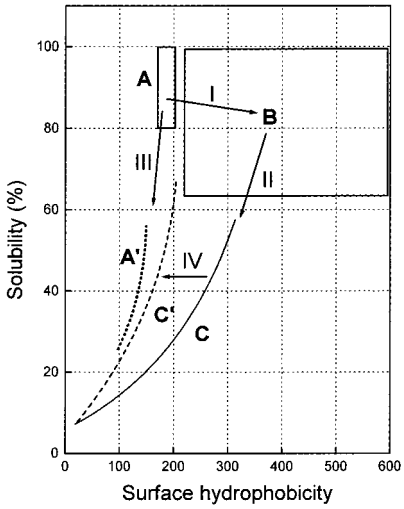

Figure 3. Proposed pathway of processes responsible for transitions between groups of commercial and laboratory soy isolates defined by their solubility and surface hydrophobicity values.

consists of denatured isolates with or without calcium ($\Delta H \approx 0$) with solubility below 70%; the other, termed A′, comprises isolates a−f and is characterized for having solubility lower than 60%, H_0 values between 60 and 160, and only partially denatured proteins ($\Delta H > 8$ J/g; DSC thermograms with the characteristic 7S and 11S peaks diminished). Most of the commercial isolates have sodium and calcium contents lower than 11 and 1.5 mg of ion/g isolate, respectively, which are values similar to those of laboratory native isolates. Isolate e has higher sodium and calcium contents (14.3 and 2.4 mg/g, respectively) and isolate f has a much higher calcium content (10.4 mg/g), and this would explain their lower solubility and surface hydrophobicity values. The higher sodium and/or calcium content in those isolates could be attributed to an intentional addition of calcium, to modified some functional properties, the use of hard water, or deficient control during precipitation−neutralization steps.

Relation between Isolate Properties and Preparation Processes. Figures 1 and 2 are combined to make Figure 3. In it, a scheme was devised to give a possible explanation of the processes leading to the preparation of isolates with such diverse characteristics. The two principal modifications of isolate protein are represented by (I) denaturation and (II) aggregation−

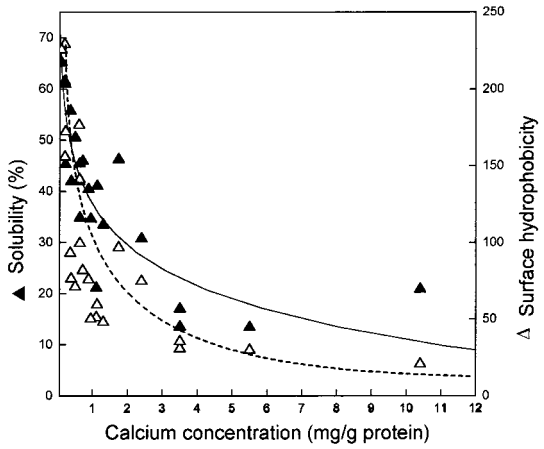

Figure 4. Effect of calcium concentration on the solubility and surface hydrophobicity of commercial soy isolates.

insolubilization. According to the proposed scheme, the native isolates (%S > 80; 170 < H_0 < 200, ΔH > 16 J/g) are converted by denaturation into totally or partially denatured isolates (H_0 > 210), but bearing high solubility when protein and calcium are not >6% and >15 mg/g of protein, respectively.

The insolubilization process of isolated proteins (process II) would be favored by the increase in protein concentration (≥8%) and/or the addition of ions (mostly divalent at a concentration >15 mg/g). In commercial isolates, the effect of spray-drying is added, which unlike freeze-drying, involves some heating. Commercial isolates are obtained with reduced solubility and surface hydrophobicity but partially denatured perhaps by the effect of mild thermal treatment before drying (group A′) or by the spray-drying process itself (process III, generally carried out at industrial scale with initial concentrations >10%). In the commercial isolates of group C′, the drying process would have a lesser effect because the isolates are already totally denatured by the preparation process. Compared to group C, the shift of this group C′ to zones of lower surface hydrophobicity could be attributed to the usual addition of phospholipids (process IV), the objective of which is to enhance the water dispersibility by increasing the surface charge (Chen and Soucie, 1985). This process would also explain the shift of group A′ to lower surface hydrophobicity zones with respect to group A.

Relation between Solubility and Surface Hydrophobicity and Calcium Content. Because the presence of salts, most specifically calcium, affects markedly isolate properties, the solubility and surface hydrophobicity of commercial soy protein isolates were plotted as a function of calcium content (Figure 4). Even when the scatter of the measured values may indicate that calcium is not the only determining factor, it can still be said that its concentration has a strong impact on the resulting solubility and surface hydrophobicity of the isolate. In tests carried out with laboratory isolates in which the only variable factor was calcium concentration, it was possible to analyze the effect of a gradual increase in the contents of this ion at low and high protein concentrations. In the low protein concentration experiments, isolates were prepared by first adding growing amounts of $CaCl_2$ to aqueous solutions (3%) of the native isolate followed by thermal treatment at 90 °C for 100 min and final dehydration by freeze-drying. To test the specific effect of calcium (added as $CaCl_2$ after the standard preparation procedure), aliquots of

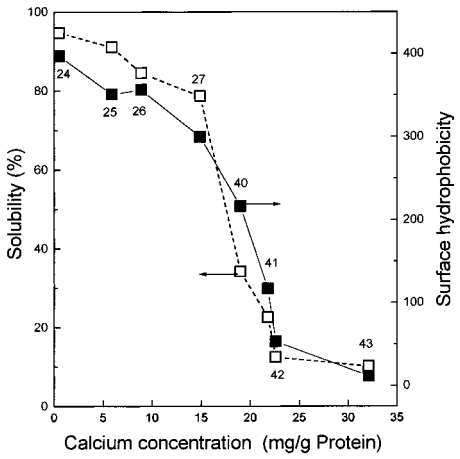

Figure 5. Effect of calcium concentration on solubility and surface hydrophobicity of laboratory soy isolates heated at low protein concentration (3%) with variable amounts of $CaCl_2$ (isolates 24−27 and 40−43, see Tables 2 and 3).

the same dispersions, but not subjected to thermal treatment, were also dehydrated (see Materials and Methods, isolates 24−27 and 40−43, Tables 2 and 3). Figure 5 shows that, provided calcium is at or below 10 mg/g, the solubility of thermally treated isolates is preserved to high values (>80%) and the surface hydrophobicity is as high (H_0 = 350−400) as that of isolates denatured at low protein concentrations without calcium addition. As calcium concentration exceeds 15 mg/g of protein, the aggregation induced by the thermal treatment becomes important, leading to a sharp decrease of solubility and H_0 (Arrese, 1991). According to Molina and Wagner (1999) protein aggregation induced by calcium was partially inhibited by the effect of chloride ion. The calcium effect on soy protein isolate would be a strong electrostatic interaction between polypeptides and hydrophobic interactions favored by the structuring water molecules (aggregation effect); the weakening of those electrostatic interactions due to chloride anion would allow an increase of solubility (aggregate dissociation effect). In commercial preparations of soy protein isolates, calcium addition is commonly carried out as $Ca(OH)_2$ during the neutralization step, instead of as $CaCl_2$ after neutralization at pH 8. Thus, commercial soy isolates would have lower solubility values than those of laboratory-prepared ones at the same calcium content. In experiments conducted in our laboratory at higher protein concentration (>10% p/v), a more pronounced decrease of solubility was observed at the same calcium content (data not shown). The combined effect of calcium [in higher degree when added in the form of $Ca(OH)_2$] and high protein concentrations (>10%) can induce considerable solubility losses in unheated isolates and more in treated ones. This observation, together with the effect of spray-drying (which includes an additional heating step), may explain the very low solubility and surface hydrophobicity presented by commercial isolates with calcium concentrations below 11 mg/g of proteins (Figure 4).

Conclusions. By studying the solubility of laboratory and commercial soy isolates, and their relation with surface hydrophobicity, it was possible to classify laboratory isolates in three groups, A−C, and commercial ones in two, A′ and C′. The groups consist of samples having proteins in different degrees of denaturation and aggregation. In laboratory isolates, these modifications were induced mainly by thermal treatments, by the effect of the medium (acid or alkaline pH, dialysis, urea, sulfite), and by the presence of calcium salts. The intimate relationship of solubility and surface hydrophobicity reinforces the importance of hydrophobic interactions in the aggregation−insolubilization process of soy proteins.

At low protein concentrations, both native and denatured isolates showed high solubility values, which could not be obtained by using commercial isolates. The marked differences between laboratory and commercial isolates regarding both solubility and surface hydrophobicity might be attributed to the additional action of industrial processes such as drying to a high protein concentration, the presence of salts added deliberately [as, for instance, $Ca(OH)_2$] or incidentally (through the water used to extract and redissolved proteins), and, besides, to phospholipid addition.

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