Water Imbibing Capacity of Soy Protein Isolates: Influence of Protein Denaturation

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In the present work the influence of the degree of protein denaturation and aggregation on water imbibing capacity (WIC) was studied. The results obtained show that the insoluble fraction of all isolates contributed the most to the WIC of the total isolate. This insoluble fraction is constituted by denaturated 11S and 7S proteins and some native 7S protein. WIC values of insoluble and soluble fractions are also influenced by the state of aggregation of their component proteins.

INTRODUCTION

Commercially produced soy protein isolates impart selected functional properties to food. The functionality of soy protein has been the subject of several studies (Hermansson, 1978; Kinsella, 1976, 1979; Johnson, 1970).

Some of the more important functional properties of soy proteins are related to their interaction with water. The water spontaneously imbibed by soy protein, as measured in this study was the water physically held within a protein matrix (Kinsella, 1976). This water produces a limited swelling of proteins (Hermansson, 1972), leading to swollen particles that do not undergo disintegration at incipient solvation. Water holding capacity is of great importance for the quality of different products, particularly doughs, batters, and comminuted meat systems.

Water imbibing capacity has been previously studied in relation to the effect of different solutes and the variation of the molar relation of 7S and 11S protein fractions (Yao et al., 1988; Lopez de Ogara et al., 1987; Flemming et al., 1974).

Prediction of hydration and aggregation properties in food systems is difficult. The most serious complication in commercial isolates arises because they may behave quite differently from native soy proteins due to processing conditions. This, in turn, causes a heterogeneous degree of denaturation and various stages of aggregation. In such complex states, molecular properties exert only a negligible direct influence, since they are overshadowed by aggregation and interaction effects.

The objective of this study was to investigate the influence of the degree of protein denaturation and aggregation on the water imbibing capacity of soy protein isolates.

MATERIALS AND METHODS

Samples. Soy protein isolates used were commercial food proteins. Type I: Samples 1–5 were isolates with less denatured proteins, (different lots of Proteinmax 90 LG from Sanbra S. A., Brazil). Type II: Samples 6–8 were isolates with totally denatured proteins but with a high water imbibing capacity (different lots of Proteinmax 90 HG from Sanbra S.A.). Type III: Samples 9 and 10 were isolates with totally denatured proteins but with a low water imbibing capacity (Proteinmax 90 HG from Sanbra S.A. and Purina protein 610 from Ralston Purina Co., respectively.

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Scheme I

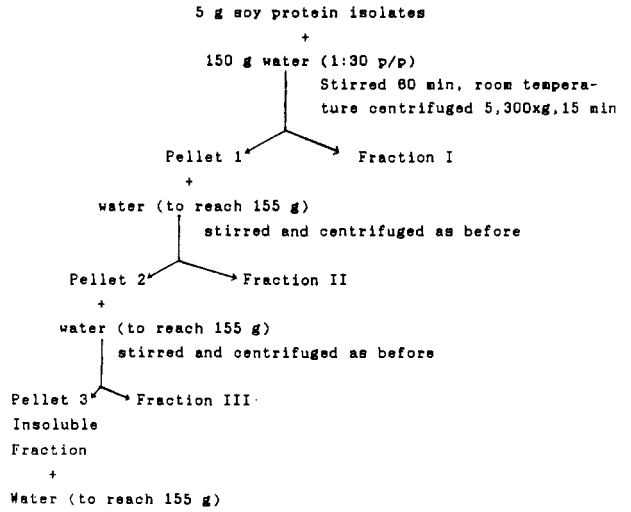

Table I. Protein Content in Extracts I–III and the Insoluble Fraction of Soy Isolates

	% protein content[a]				
	extracts				
isolate	I	II	III	Σ extracts	insoluble fraction
1	35.7	18.5	12.4	66.6	33.4
2	35.4	17.4	13.0	66.2	33.8
3	14.5	4.9	3.6	23.0	77.0
4	25.0	6.1	8.3	39.4	60.6
5	12.6	10.1	12.2	34.9	65.1
6	20.6	4.3	3.9	27.8	72.2
7	25.6	9.4	4.8	39.7	60.3
8	14.4	4.4	1.2	20.0	80.0
9	48.9	14.7	7.0	70.7	29.3
10	10.1	3.4	0.9	14.4	85.6

[a] Protein contents were determined by Biuret method. Values are expressed as the percent of protein with respect to total protein of the isolate. Each value is the result of three determinations.

The pH of 1% dispersions in water was 6.8–7.0. The protein and moisture contents of soy protein isolates were approximately 90% (N × 6.25) and 5%, respectively.

Protein Extracts. Proteins were extracted from soy isolates with water as shown in Scheme I. Determination of protein content by the Biuret method (Gornall et al., 1949) was performed on aliquots of extracts I–III. A standard curve was obtained by using crystalline bovine serum albumin (BSA). The remainder of these fractions and aliquots of the 3% dispersions in water of the different soy protein isolates used were lyophilized and dis-

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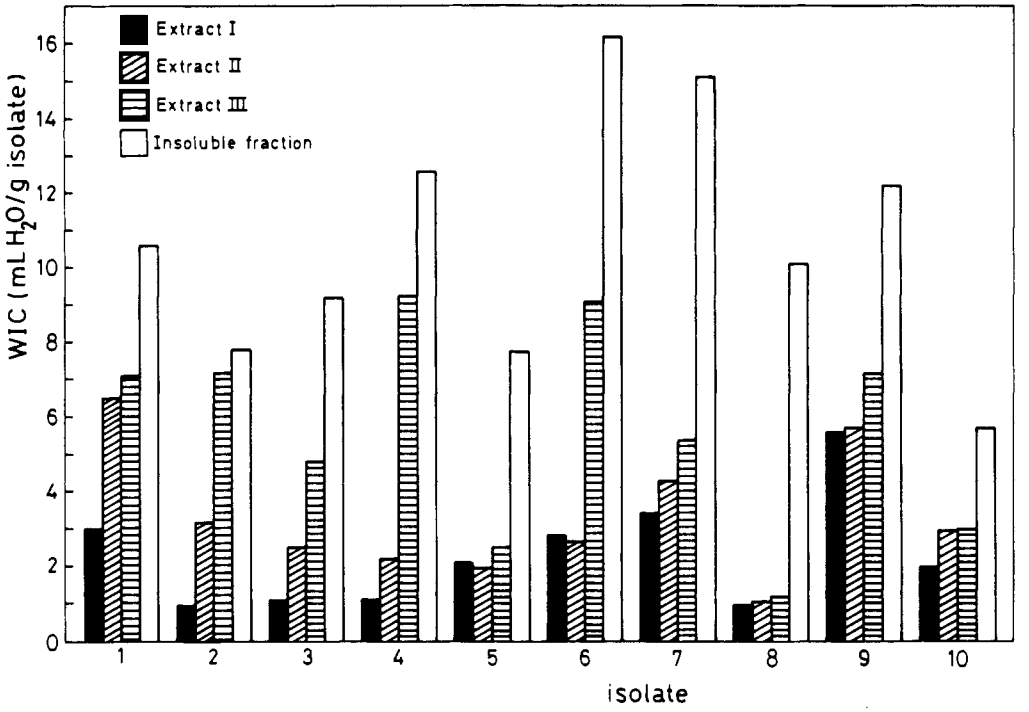

Figure 1. Water imbibing capacity (WIC) of lyophilized fractions of soy isolates.

aggregated in a mortar to obtain a fine powder; 3% dispersions of total isolates or extracts in water were lyophilized in a Thermovac Industries Corp. freeze-dryer.

Water-Imbibing Capacity (WIC). WIC values of soy protein isolated were determined by 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 a wetted filter paper which was fastened to a glass filter placed 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 isolated. Determinations were performed in duplicate.

Electrophoresis (SDS-PAGE). Slab sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the Laemmli discontinuous buffer system (Laemmli, 1970) at a gel concentration of 12.6% using Pharmacia gel electrophoresis apparatus GE-2/4. Sample preparation for SDS–PAGE was carried out as follows: 300-mg protein samples were dissolved in 30 mL of 0.086 M Tris, 0.09 M glycine, and 4 mM EDTA, pH 8 buffer, containing 8 M urea. The dispersions were mixed with an equal volume of SDS–PAGE sample buffer containing 5% (v/v) 2-mercaptoethanol. About 50 mg of protein was applied to each gel slot. Gel slabs were fixed and stained simultaneously in a solution of methanol, acetic acid, and water (5:5:2) and 0.1% Coomassie Brilliant Blue R-250. Molecular weights of the protein bands were estimated by means of the MW-SDS-70L Pharmacia kit. Analyses were done in duplicate.

Differential Scanning Calorimetry (DSC). Samples (15–20 mg) of 20% dispersions in water were hermetically sealed in aluminum pans. An empty double pan was used as reference. The samples were analyzed at 10 °C/min in a Du Pont Model 910 instrument attached to a Hewlett-Packard 7046 B recorder. After DSC run, the capsules were punctured and the dry matter was determined by drying overnight at 105 °C. Temperature calibration and the cell constant were determined by using indium. 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.

The areas per unit weight for each peak (partial area cm^2/total mg of dry matter) were also determined.

Turbidity. The turbidity was measured as the absorbance at 600 mm of successive water dilutions of each fraction.

Statistical Analysis. Variance analysis of two factors and test $LSD_{0.05}$ for media comparison were used.

RESULTS AND DISCUSSION

Table I shows the percentage of protein extracted with distilled water by successive extractions of the analyzed commercial soy isolates. The percentage of insoluble (nonextractable protein) was calculated as the difference between the total protein percentage and the soluble protein percentage. It can be seen that although there are differences between the percentages of total protein extracted by each extraction from the different isolates (except in the case of isolate 5), most of the water-soluble protein was extracted in the first extraction.

Table II includes the WIC values of both the nonlyophilized and lyophilized total isolate and the sum total of the contributions of all fractions (extractable and insoluble) to the total WIC. It can be seen that lyophilization alters the WIC values by a percentage not higher than 25%. These significant differences ($LSD_{0.05}$ 0.10) can be attributed not only to lyophilization but also to the further grinding (used to prepare isolates for WIC analysis) which alters the particle size of the powder obtained. The modifications of the WIC values attributed to lyophilization and grinding procedures do not alter the relative behavior of the samples.

WIC analysis of the soluble and insoluble lyophilized fractions (Figure 1) shows that the insoluble fraction of all isolates is that containing the highest WIC—and therefore contributing the most to the WIC of the total isolate (Figure 2). This contribution is not so evident in samples 1, 2, and 9 because of their low content of insoluble protein.

When the total WIC is calculated as resulting from the contributions of each fraction, values similar to those obtained by direct measurement of WIC on the isolate are obtained (Table II). This leads to the conclusion that protein behavior is similar—as far as water absorption capacity is concerned—in the total isolate and in the different fractions obtained according to their solubilities.

The extent of protein denaturation was analyzed by means of differential scanning calorimetry (DSC) to explain the variations of WIC of the different fractions. Calorimetric studies showed that isolates 1–5 have partially

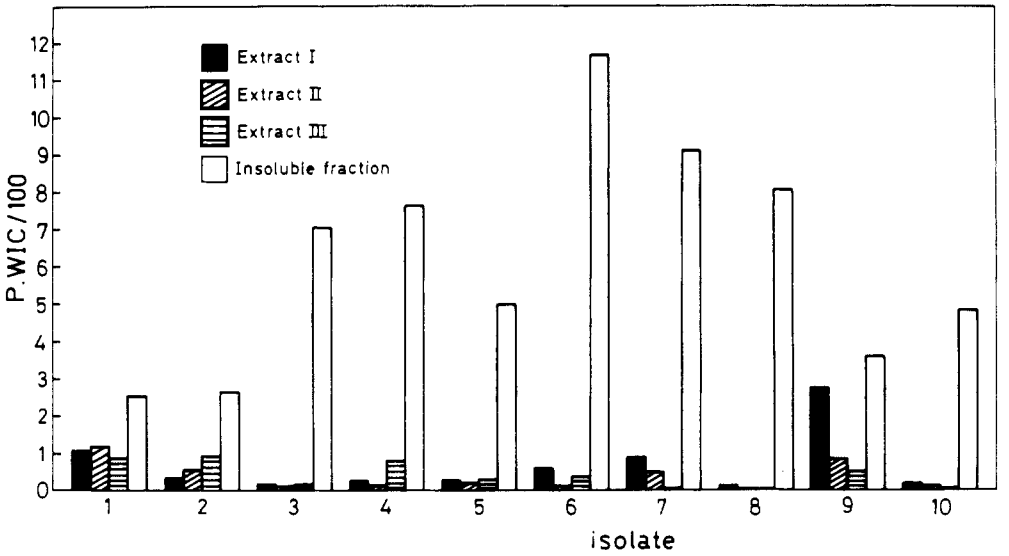

Figure 2. Contribution of the WIC of each fraction to the total WIC. P is the percent protein content of extracts I–III and the insoluble fraction. WIC is the water imbibing capacity of each fraction expressed in milliliters of H_2O per gram of sample.

Table II. Water Imbibing Capacity (WIC) of Soy Isolate, Lyophilized Soy Isolate, and Total Contribution of Different Fractions

	WIC[a]		
sample	soy isolate	lyophilized soy isolate	$\sum$[b]
1	5.7	5.9	6.7
2	6.0	4.6	4.4
3	5.8	7.0	7.5
4	8.2	6.9	8.8
5	5.3	5.1	5.8
6	11.3	8.8	12.8
7	9.6	8.6	10.7
8	9.1	7.1	8.3
9	7.1	6.4	7.7
10	5.9	5.2	5.2

[a] Results are expressed as milliliters of water per gram of sample. [b] $\sum = (P_I WIC_I + P_{II} WIC_{II} + P_{III} WIC_{III} + P_{INS} WIC_{INS})/100$, where P is the percent protein content of extracts I–III and the insoluble fraction (see Table I). $LSD_{0.05}$ between treatments, 0.10; $LSD_{0.05}$ between samples, 0.23.

denatured proteins (ΔH (J/g) = 6.3, 5.4, 3.3, 5.0, and 6.7 J/g, respectively). Instead, samples 6–10 showed no endothermic peaks (ΔH = 0 J/g). This latter results shows that these isolates contained completely denatured proteins.

In addition to the total lylophilized isolates, extracts I–III and the insoluble fraction were analyzed by DSC in those isolates in which $\Delta H > 0$. The general thermal behavior of these isolates can be seen in Figure 3. Thermal denaturation thermograms of the total isolates exhibit the two characteristic peaks corresponding to endothermic transitions of the 7S (peak A) and 11S (peak B) proteins with the temperatures of T_{max} peak 74 and 86 °C, respectively. These results are in agreement with those reported by other authors (Hermansson, 1978; Wagner and Añón, 1990; Arrese et al., 1989). Thermograms of the various extracts differ both in T_{max} and in total area. There is also a shift of T_{max} toward higher values, which can be attributed to a salt effect (Hermansson, 1978; Damodoran and Kinsella, 1982) on the basis of the following facts: this shift is more pronounced in extract I, which contains the higher salt content. This shift is absent when the salts are eliminated from the extracts by previous dialysis three times against 20 volumes of distilled water.

Thermogram of extract I shows the largest area, which decreases gradually in the following extracts, the smallest area corresponding to the insoluble fraction. These results

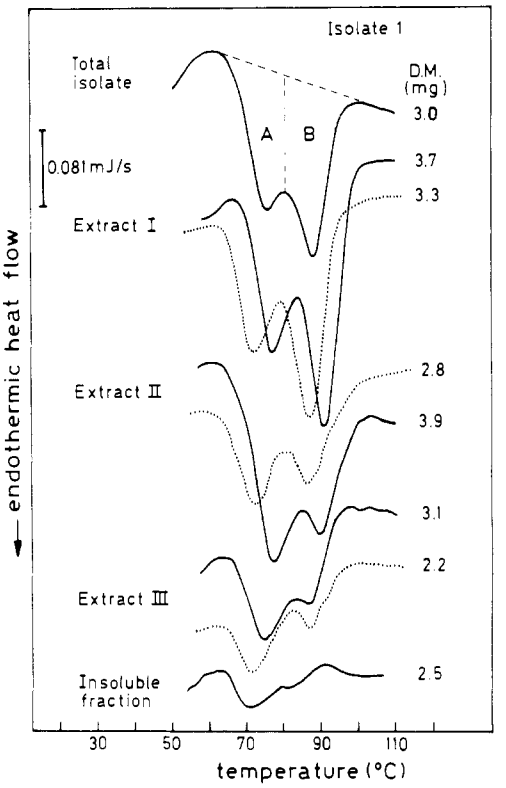

Figure 3. DSC thermograms of 20% dispersion of the sample (isolate and different fractions) in distilled water. Heating rate 10 °C/min. (—) Without dialysis; (···) dialyzed against distilled water (three times, 20 volumes each); DM, dry matter. Peaks A and B correspond to thermal denaturation of 7S and 11S proteins, respectively.

indicate that the more native proteins are solubilized mainly in the first washing. The variations of the abovementioned areas are due mainly to the obvious decrease of peak B and lead to its total absence in the insoluble fraction. Peak A also decreases in the successive extractions but to a lesser extent than peak B. This effect can be quantitated by analysis of both the total specific areas and the areas corresponding to each peak, in the thermograms of each isolate (Figure 4). The total areas of the thermograms were divided into partial areas corresponding to each endothermic transition, thus making possible the calculation not only of the total enthalpy of dena-

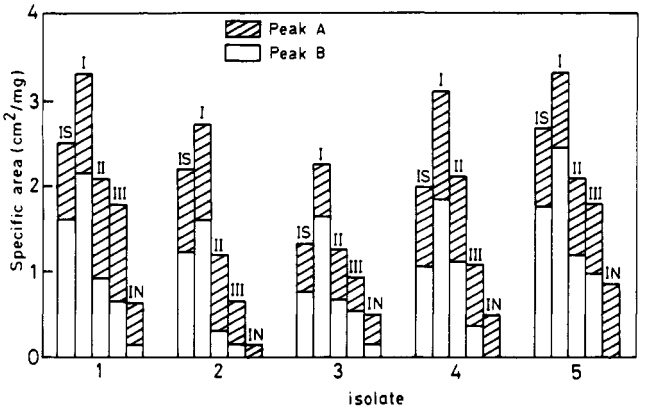

Figure 4. Specific areas corresponding to DSC thermograms of lyophilized isolates and different fractions of each sample. IS, isolates; I, II, III, fractions I–III, respectively; IN, insoluble fraction.

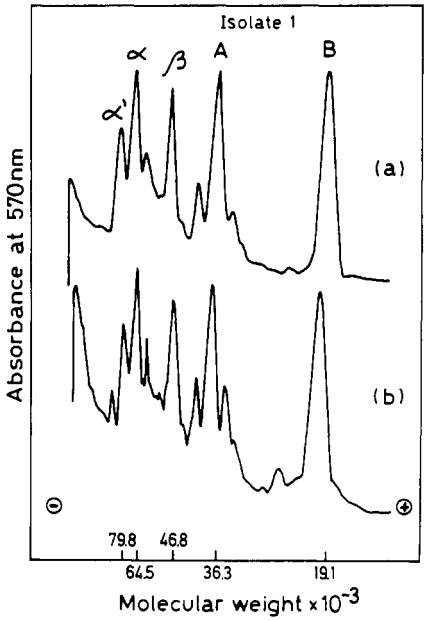

Figure 5. Densitometric scans of the SDS gel electrophoretic patterns. (a) Total protein isolate; (b) water-insoluble fraction.

turation (ΔH) but also of the areas of peaks A and B. As the peaks associated with the different transitions overlap and their shapes are not known, only crude estimates of the areas are possible. However, information can be obtained from these estimations. These results indicate that in the insoluble fraction there is practically no native protein 11S, but some native 7S protein does exist. This suggests that protein 11S has a greater tendency to form insoluble aggregates when it reaches an advanced extent of denaturation; instead, protein 7S can form this type of aggregate even if it is not in a completely denatured structure. This is in agreement with other papers in which it is stated that protein 7S has a higher tendency to aggregate than protein 11S (Saio et al., 1974; Hashizume et al., 1975).

PAGE results (Figure 5) eliminate the possibility that 11S protein would be absent in the insoluble fraction for solubilization in successive extractions. The SDS–PAGE patterns of total isolate and insoluble fraction of all isolates show approximately the same relative composition of the two major soybean protein components, 7S and 11S, and of their subunits α, α', β and polypeptides A and B, respectively.

In those samples having $\Delta H > 0$, the increase of the proportion of denatured protein (indicated by the decrease

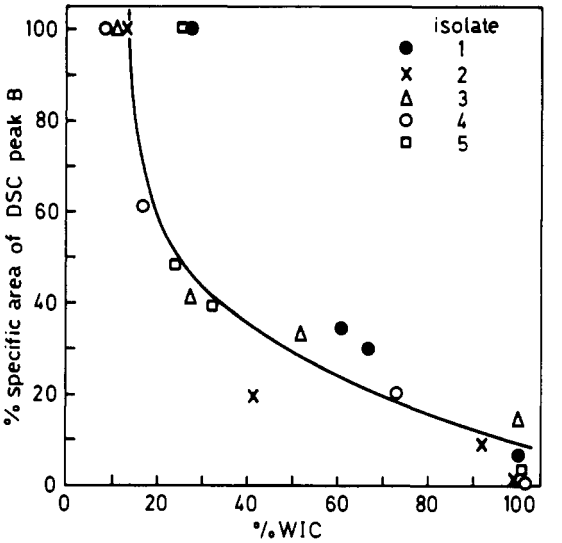

Figure 6. Relationship between the specific area of DSC thermogram peak B and WIC of different fractions (I–III and insoluble). Peak B specific area was expressed as percent with respect to the specific area of peak B of fraction I (maximal value). WIC was expressed as percent with respect to WIC value of insoluble fraction (maximal value).

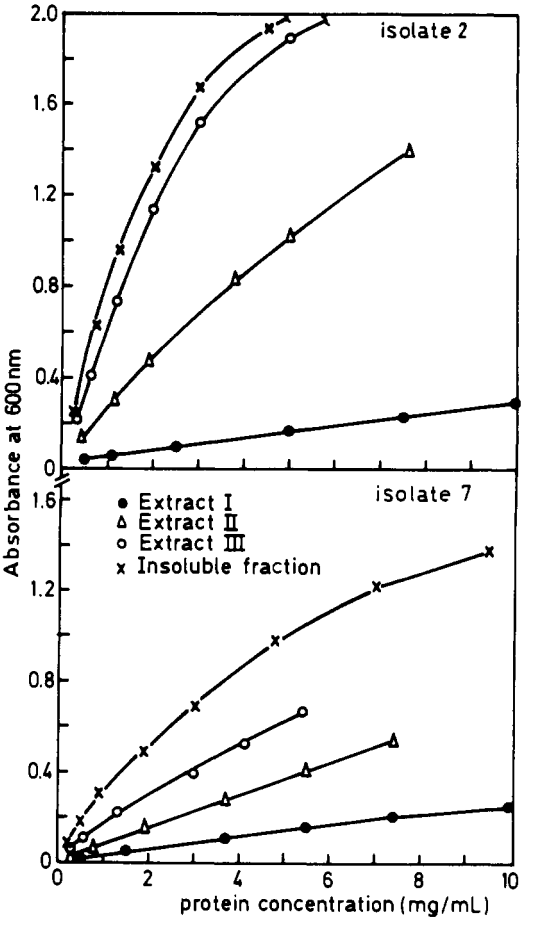

Figure 7. Turbidity as a function of protein concentration of successive water dilutions of each fraction.

of the specific areas of each DSC peak) (Figure 4) is accompanied by a correlative increase of the WIC values (Figure 1). In those samples having $\Delta H > 0$, the increase of the proportion of denatured protein present in the range from soluble to insoluble fractions (Figure 4) is accompanied by a correlative increase of the WIC values (Figure 1). This can be seen more clearly if specific area values

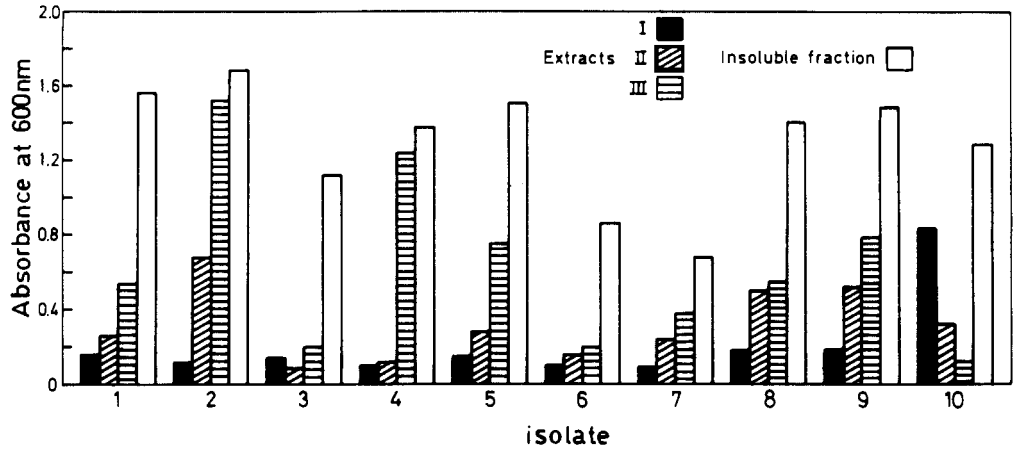

Figure 8. Turbidity at a fixed protein concentration (3 mg/mL) of the different fractions of each isolate. Values were obtained from the plots of turbidity vs protein concentration (Figure 6).

of both DSC peak B and WIC are plotted as percentages of the respective maximum values (Figure 6).

It was concluded from these results that the insoluble protein fraction contributes a larger extent to WIC, as a consequence of its having proteins in advanced denatured state. This behavior of the different fractions with regard to WIC is also observed in isolates having $\Delta H = 0$ (Figure 1, isolates 6–10). This fact cannot be explained by taking only the degree of denaturation into account, since all the fractions are formed by denatured proteins.

As we have previously reported, completely denatured proteins have a higher tendency to aggregate at higher surface hydrophobicity (Wagner and Añón, 1990). Furthermore, there is a tendency for solubility to decrease with an increase in the free sulfhydryl content (Wagner and Añón, 1990). This led us to think that the differences in WIC of both the insoluble and soluble fractions were also influenced by the state of aggregation of their component proteins.

The turbidity method is suitable to assess the state of aggregation of proteins in solution. As an example, Figure 7 shows the results obtained by measuring the absorbance at 600 nm of several serial dilutions of the different fractions of several isolates studied. As it can be seen in Figure 8, turbidities of practically all samples (for the same protein concentration) increase gradually from extract I to the insoluble fraction. These results could be explained by taking into consideration that in extracts I–III both soluble proteins and those forming small and easily dispersable aggregates are present. The increase of the turbidity in successive extractions could be attributed to the augmentation of the number and/or size of the aggregates. Further experiments are necessary to confirm this statement.

Comparison between Figures 1 and 8 shows that there is a correlation in each isolate between the WIC values and the presence of aggregates (as evidenced by turbidity). Both parameters increase toward the insoluble fraction. If the different samples are compared, however, it can be seen that those samples in which their insoluble fractions have the higher WIC values (isolates 6 and 7) exhibit lower turbidities than the equivalent samples of the rest of the isolates. This suggests that not only the degree of aggregation but also the aggregate size plays a role. If larger particles would be present in isolates 6 and 7, leading consequently to a lower number of them, it could explain why even though they have a high water absorption capacity, turbidity values remain low.

CONCLUSIONS

The present work has demonstrated that the water imbibing capacity (WIC) of the studied commercial soy isolates is the sum of the contributions of the dispersable and insoluble proteins. As expected, the insoluble protein fraction contributes to the larger extent on WIC of the total isolate. Some commercial soy protein isolates are subjected to a thermal treatment during processing before the spray-drying step that leads to different denaturation degrees of their proteins. The advanced denatured state of 7S and 11S proteins in the insoluble fraction leads to the formation of aggregates that increase the water absorbing and retaining capacity. 11S protein has a greater tendency to form insoluble aggregates when it reaches an advanced extent of denaturation. 7S protein is capable of aggregation even in a not totally denatured state.

The studied property depends not only on the denaturation state but also on the degree and type of protein aggregation as becomes evident from the WIC values of the different fractions of totally denaturated isolates.

ACKNOWLEDGMENT

E.L.A. is a fellow of the Comision de Investigaciones Científicas de la Provincia de Buenos Aires (CIC). J.R.W. and M.C.A. are members of the Research Carreer of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). D.A.S. is a full professor of the Facultad de Ciencias Exactas—Universidad Nacional de la Plata. This work was supported by grants from CONICET and CIC.

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Received for review August 13, 1990. Revised manuscript received March 11, 1991. Accepted March 18, 1991.