Effect of Physical and Chemical Factors on Rheological Behavior of Commercial Soy Protein Isolates: Protein Concentration, Water Imbibing Capacity, Salt Addition, and Thermal Treatment

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The influence of different factors on viscosity and rheological behavior of commercial soy isolates was studied. Water imbibing capacity and protein concentration are interdependent parameters which define the viscosity. Groups of isolates determined by differences in the degree of denaturation and aggregation due to processing treatments, as reflected in viscosity and different pseudoplastic behaviors, show significant correlation with those groups based on functional and structural properties or their response to NaCl or Na_2SO_3 addition. The decrease in viscosity due to salt addition is highest in denatured samples with low calcium content. Thermal treatments lead to more viscous dispersions in isolates with partially or even totally denatured proteins. The increase in viscosity is observed even in dispersions with viscosity previously decreased by salt addition.

INTRODUCTION

Soybean protein isolates have high nutritional value and their consumption has increased steadily (Mohri and Matsushita, 1984).

A number of papers have been published on processing treatments that may change the product characteristics and use in food applications of soy proteins (Rakosky, 1970; Circle and Smith, 1972; Wu and Inglett, 1974; Kinsella, 1976; Ohren, 1981), but the relationship between functional properties and the performance in food systems remains obscure (Hermansson and Akesson, 1975; Lopez de Ogara et al., 1986).

Much remains to be accomplished in the application of fundamental physicochemical information to protein modification and process parameter optimization to design protein isolates with specific functional attributes. This would be very useful for their successful utilization in various food systems.

Knowledge of viscosity and rheological properties allows one to study conformation and interaction of molecules and provides a tool for process monitoring and control.

Although prediction of hydration and aggregation properties is not yet possible, rheology is often the only method that may be used to describe with confidence the state or performance of such complex food systems as protein isolates (Rha and Pradipasena, 1986).

The aim of this study is to analyze the physicochemical factors which determine the viscosity and rheological behavior of several commercial isolate dispersions. These are constituted by proteins with different states of denaturation and aggregation due to thermal and/or chemical treatments during their isolation and processing.

The effect of important parameters such as water imbibing capacity, salt concentration, and thermal treatment on viscosity has been investigated.

MATERIALS AND METHODS

Samples. The soy protein isolates used were commercial food proteins; samples 1–14 were produced by Sanbra S.A., Brazil, and 15–19 by Ralston Purina, U.S.A.

Group I included isolates 1–5, Proteimax 90 LG, with less denatured proteins.

Group II included isolates 6–12, Proteimax 90 HG, with totally denatured proteins by thermal treatment.

Group III included isolates 13 and 14, Proteimax 90 MP; isolates 15 and 16, Supro 500 E; isolate 17, Supro 610; isolate 18, Supro 515; and isolate 19, Supro 590, with totally denatured proteins by thermal and reducing treatments.

The pH of 1% dispersions in distilled water was 6.8–7.0. The protein and moisture contents were 76–82% (N × 5.7; Mossé, 1990) and 5.0–6.5%, respectively.

Protein Solubility. Solubility was determined as the nitrogen solubility index (NSI) according to AACC (1983) Method 46-23. This assay was carried out in water at pH 7.0.

Water Imbibing Capacity. WIC of soy protein isolates was determined using a modification of the Baumann apparatus (Torgensen and Toledo, 1977) as previously described (Sorgentini et al., 1991). It is expressed as milliliters of water imbibed per gram of isolate.

Imbibing Water Ratio. Ratios of total to imbibed (T/I) water (Urbanski et al., 1983) were calculated as

$$\frac{T}{I} = \frac{\text{g of total water/g of dispersion}}{\text{g of imbibed water/g of dispersion}}$$

Sulfhydryl Content. Free sulfhydryl groups were determined according to the Beveridge et al. (1984) method, with slight modifications, as described previously (Wagner and Añón, 1990).

Differential Scanning Calorimetry (DSC). The samples were analyzed in a Du Pont Model 910 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 three times.

Apparent Viscosity (η_{app}). Apparent viscosities were measured in isolate dispersions between 1.6 and 14% (w/w). Measurements were carried out at 20 °C in a Haake Rotavisco RV2 viscometer using a sensor system NV and a rotor speed varying from 0 to 128 rpm in 2 min and kept 1 min at maximal speed.

Apparent viscosity was calculated as

$$\eta_{app} = GS/n \text{ (cP)}$$

where G is an instrument factor [cP/scale grade min], S is the scale value, and n is the rotor speed (rpm).

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Table I. Apparent Viscosity (η_{app}) of 10% (w/w) Isolate Dispersions Measured at 128 rpm, Water Imbibing Capacity (WIC), Total Denaturation Enthalpy (ΔH), Sulfhydryl Group Content (SH), Solubility in Distilled Water (NSI), and Calcium Ion Content (Ca^{2+}) of Soy Isolates

isolate	η_{app}, cP	WIC, mL/g	ΔH cal/g	SH, μmol/g	NSI, g/100 g	Ca^{2+}, mg/g
1	48.8	5.8	1.5	0.20	52.9	0.62
2	47.5	6.2	1.3	0.32	42.3	0.62
3	61.7	7.8	1.2	0.09	29.8	0.64
4	45.0	5.2	1.6	0.18	51.6	0.22
5	29.6	6.0	0.8	0.17	22.8	2.40
6	141.4	12.2	0	0.04	27.9	0.36
7	109.2	11.0	0	0.18	24.5	0.71
8	141.4	10.6	0	0.25	22.7	0.88
9	95.6	9.4	0	0.49	21.3	0.50
10	131.1	12.8	0	0.75	22.9	0.38
11	154.2	11.6	0	0.03	46.7	0.20
12	73.2	7.3	0	2.32	15.3	1.10
13	61.7	7.3	0	3.39	29.0	1.66
14	64.3	7.6	0	1.13	17.8	1.13
15	52.7	8.8	0	2.06	9.2	1.50
16	59.1	7.8	0	4.76	10.6	1.53
17	33.4	6.4	0	4.71	8.9	1.18
18	65.5	8.1	0	2.43	15.0	1.14
19	55.3	8.0	0	2.65	14.4	1.30

Shear stress was calculated as

$$\tau = AS \text{ (dyn/cm}^2\text{)}$$

where A is an instrument factor [dyn/cm^2 scale grade].

For sensor system NV, G = 329 cP/scale grade min and A = 17.8 dyn/cm^2 scale grade.

Calcium Determination. Three grams of isolates was dry-ashed at 600 °C in a muffle oven. After cooling, the ashed samples were dissolved in 5 mL of concentrated HCl and diluted to 100 mL with distilled water. The calcium concentration in diluted samples was determined by emission spectroscopy using argon plasma (IL Model 300 spectrometer; λ = 422.67 nm).

RESULTS AND DISCUSSION

Three different groups of commercial soy isolates were defined, on the basis of data detailed in Table I: group I, samples 1–5, with proteins partially denatured ($\Delta H > 0$), with low values of apparent viscosity (between 30 and 60 cP), and with values of WIC of 5–8 g of H_2O/g of protein; group II, samples 6–11, having totally denatured proteins ($\Delta H = 0$), with higher values of apparent viscosity and WIC (>96 cP and >9 g of H_2O/g of protein, respectively). Group III, samples 12–19, which have totally denatured proteins ($\Delta H = 0$) similar to those of group II, but their values of WIC are as low as those of group I. They differ from those two groups in the high content of calcium ion and free sulfhydryl groups.

These three groups, which show differences in functional and structural properties as well as in calcium ion content, agree with the groups defined under Materials and Methods in relation to the previous treatments during sample processing, except sample 12, which is included in group III. It is a lot of Proteimax 90 HG with higher calcium content than all other samples of this type.

So, it is evident that functional behavior of commercial isolates depends on manufacturing process. However, the demand from industry to predict the functionality of food proteins has not yet been satisfied. This study has examined the influence of different factors on flow properties of protein dispersions, since these provide a key for modifications to impart desired functionality.

Concentration Dependence. Figure 1 shows the flow curves for different dispersions of soy protein isolates. As protein concentration increases, there is an increase in shear stress over the entire range of shear rates measured in samples of any group.

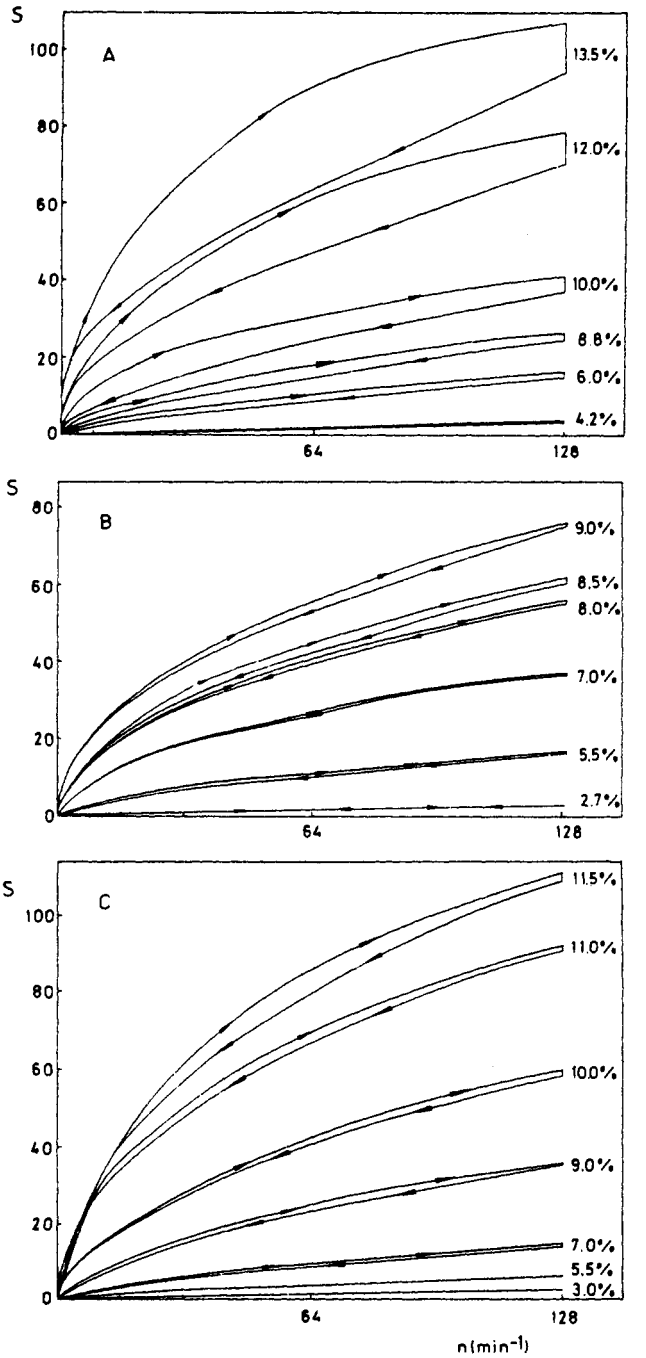

Figure 1. Flow curves of soy protein isolate dispersions in distilled water at different concentrations. (A) Group I, sample 5; (B) group II, sample 6; (C) group III, sample 12. S, scale grade value; n, rotor speed (rpm).

The apparent viscosity reflects the effects of the individual protein molecules in very dilute protein dispersions. As the concentration is increased, protein–protein interactions become dominant and more molecular water is immobilized, resulting in the additional increase of apparent viscosity at high concentration (Frisch and Shima, 1956). This may account for the progressive increase in apparent viscosity of soy protein isolate dispersions with increasing protein concentration.

All samples have pseudoplastic flow behavior, as previously reported (Circle et al., 1964; Hermansson, 1975). Apparent viscosity was not directly proportional to shear rate.

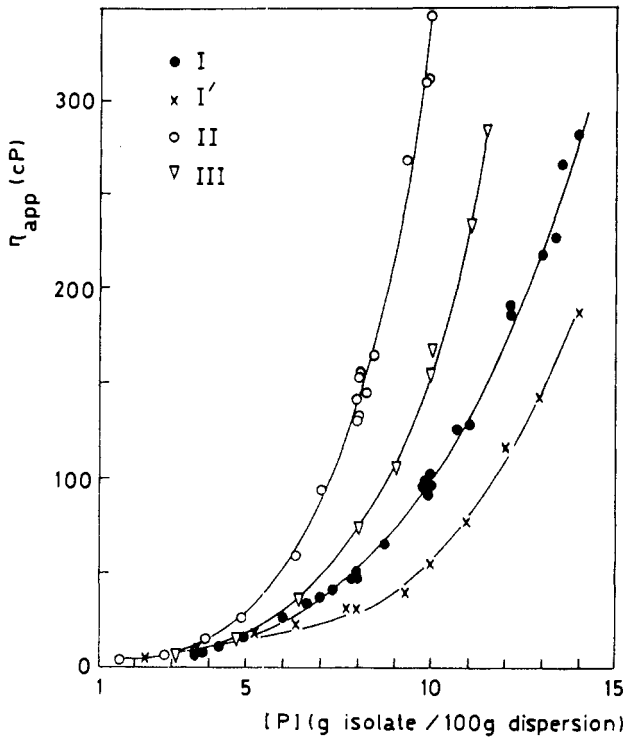

Figure 2. Effect of protein concentration on apparent viscosity (η_{app}) of soy protein isolate dispersions. Rate of shear, 128 rpm; temperature, 20 °C.

Samples of group I showed a time-dependent flow property and behaved thixotropically even at low concentration values such as 6% (Figure 1A). At higher concentrations they showed considerable thixotropy.

In group II and III samples (Figure 1B,C) the hysteresis loops typical of the suspending systems of macromolecules were found at higher concentrations (about 8–9%) and their areas were smaller than those of group I.

Changes in shape and in interactions between particles may arise from unfolding as a result of denaturation or from deformation of hydrated aggregates or molecules along the stream line by shearing stress (Pradipasena and Rha, 1977; Lee and Rha, 1977, 1979). Those factors, in addition to particle size, are responsible for the η_{app} values and the hysteresis effect.

Thixotropy could be caused by the breaking or deformation of hydrated aggregates, since the concentration was high enough for the existence of proteins in aggregate form in most dispersions. It also could arise from unfolding as a result of total denaturation of proteins in group I. In groups II and III proteins were totally denatured and could be previously associated in more stable aggregates that were not easily broken by the shear stress. Since η_{app} values between these last two groups are quite different, other factors may be evolved.

When samples are non-Newtonian, η_{app} values are highly dependent on the shear rate at which shear stress is measured (Figure 1). To have comparative results, values of η_{app} of isolates of each group at 128 rpm shear rate were plotted as a function of protein concentration (Figure 2).

Data of all samples in each group fell on the same curve (group I, curve I; group II, curve II; group III, curve III), sample 5 of group I being the only exception. A different curve, with lower values of η_{app} than those of group I (in all protein concentrations), was observed (see curve I′). The difference between this sample and all others in that group is the high calcium ion content (see Table I).

When samples with $\Delta H = 0$ are compared, it can be seen that all those with free SH groups <1 μmol/g and calcium

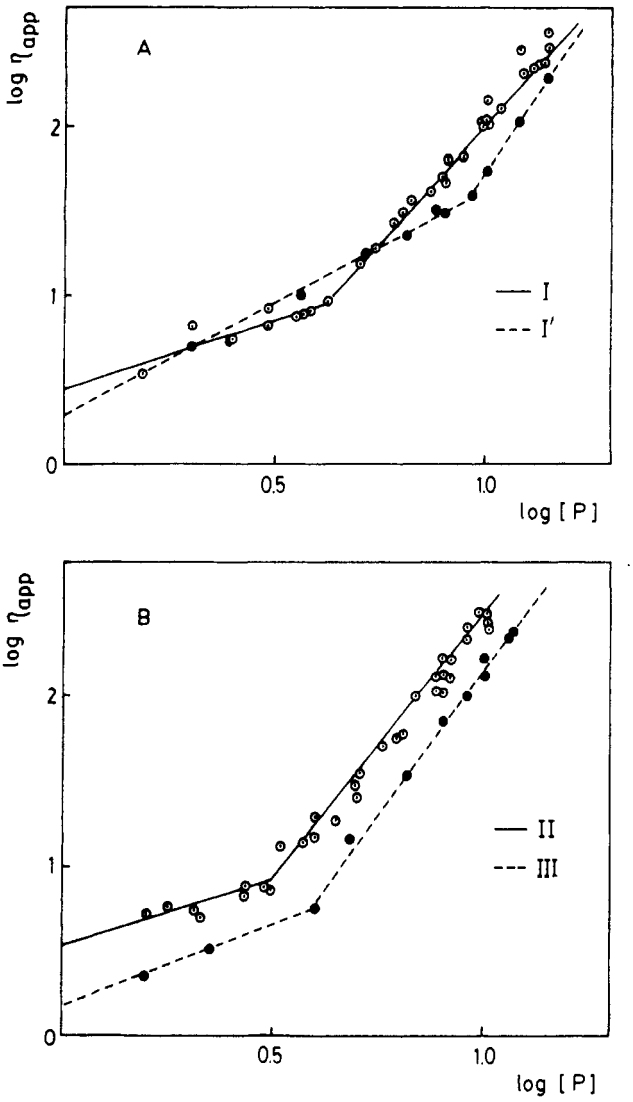

Figure 3. Double-logarithmic representation of curves in Figure 2. The system is represented by the model $y = b_0 + b_1x + b_2(x - x_0)$, for $x > x_0$. (A) Isolates of groups I (⊙), $r^2 = 0.999$, and I′ (●), $r^2 = 1.000$. (B) Isolates of groups II (⊙), $r^2 = 0.997$, and III (●), $r^2 = 0.999$. (—) Low calcium ion content. (- - -) high calcium ion content.

ion concentration <1 mg/g show similar behavior (group II). Although all other samples (group III) have also $\Delta H = 0$, they show viscosity values similar to those of samples of group I, with $\Delta H > 0$. This is so because, in addition to thermal denaturation, they suffered other treatments during their processing [S–S bridge reduction and addition of calcium salts (Table I)].

While protein aggregation caused by added calcium ions is generally understood as intermolecular ionic cross-linking (Saio et al., 1968), the aggregation of dispersed soybean protein due to heat treatment involves nonspecific interactions, including hydrophobic association. These associations of protein molecules contribute significantly to the size and shape of the dispersed protein and the shear and time dependence of the protein dispersions (Rha and Pradipasena, 1986).

To start interactions between calcium ion promoted aggregates, a highest protein concentration seems to be required.

$CaCl_2$ increases the positive charge on a protein through the binding of calcium ion (Mulvihill and Kinsella, 1988; Arntfield et al., 1990). This change in net charge resulted in increasing repulsive forces and destabilization of

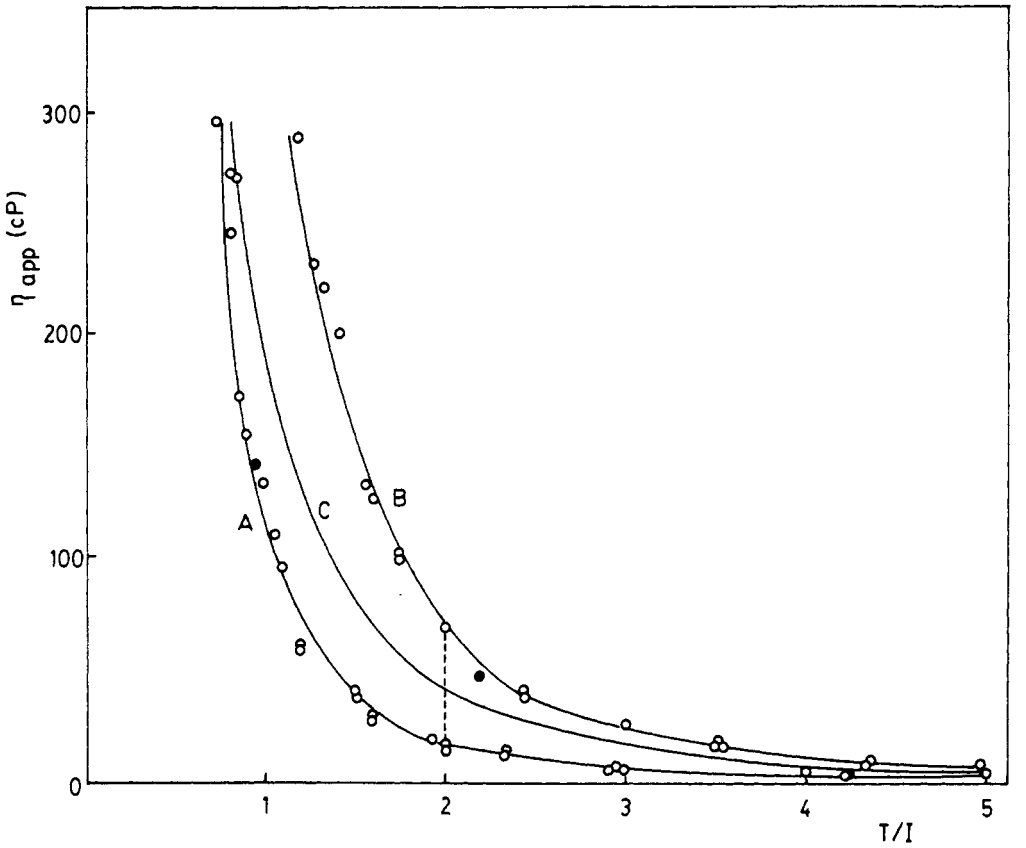

Figure 4. Relationship between apparent viscosity (η_{app}) and imbibed water ratio (T/I) for commercial soy protein isolates. T, grams of total water per gram of dispersion; I, grams of imbibed water per gram of dispersion. (A) Representative isolate of group II (sample 6). (B) representative isolate of group I (sample 5). (C) Theoretical curve, $r^2 = 0.895$; number of determinations, 121. (- - -) Line of iso-T/I relationship; (●) points of isoconcentration (8% w/w).

proteins. There is a point, however, where the masking of the net charge repulsion is the dominant factor and the aggregate formation is accelerated (Nakamura et al., 1978; Varunsatian et al., 1983).

All curves of Figure 2 showed an exponential increase in the η_{app} with increase in concentration. As already stated (Holdsworth, 1971; Hermansson, 1975; Holmes et al., 1977), the rheology of a soy protein dispersion can be expressed by the power law.

It also satisfies the equation

$$\eta_{app} = \eta_0 + a[P]^n$$

where η_0 is the initial viscosity, [P] is the isolate concentration in the protein dispersion, and a and n are constants whose values depend on the nature of the polymer and solvent and on the temperature.

Double-logarithmic representation of curves in Figure 2 was plotted in Figure 3. The following can be seen:

(a) Samples of group II (Figure 3B) showed a slightly steeper slope than those of group I (Figure 3A). In those samples with higher degree of denaturation there is a greater influence of concentration on η_{app}. This is so because of a major protein–protein interaction due to an expansion in the dimensions of the protein coil (Rha and Pradipasena, 1986).

(b) The degree of interaction between proteins is significant for samples of group II at value higher than log [P] = 0.5 ([P] ≃ 3%). For samples of group I the interaction becomes evident only when values higher than log [P] = 0.632 ([P] ~ 4%) are reached.

(c) In both cases there is an abrupt change in the slopes of the lines at lower values of log [P]. In more dilute solutions the slope is less steep. The influence of increasing concentration was negligible. The proteins have a quasi-Newtonian behavior; there is practically no interaction between them (Rha and Pradipasena, 1986).

(d) Sample of group I ($\Delta H > 0$) with high calcium ion concentration may be considered as a subgroup (I', Figure 3A). It is known, from previous work in our laboratory, that the aggregation induced for those ions is reflected in low solubility and hydrophobicity (unpublished data: average total hydrophobicity of group I = 1.87 μg of SDS/500 μg of protein and of group I' = 0.92 μg of SDS/500 μg of protein).

The displacement of the point of inflection observed in Figure 3A may be caused by a poor capacity of calcium ion promoted aggregates to interact with each other. A higher protein concentration would be required for interactions between the suspended particles to start affecting the correlation η_{app} vs protein concentration.

(e) The same effect of calcium ion observed in native samples is observed in samples with $\Delta H = 0$.

Representative samples of group III, with high calcium ion concentration, showed a similar displacement of the line, without evident change in the slope.

These samples have, in addition, a high free sulfhydryl group content. The treatment of those samples during reduction processing affects their WIC values (all aggregates in this group have lower imbibing capacity than those of group II). It might be due to a change in protein conformation that could have altered the hydrophilic interactions between proteins and water molecules (Yao et al., 1988; Dosaka et al., 1979).

WIC Influence on Viscosity. The viscosity of all samples is related, at least partially, to WIC values. Samples with higher WIC values also show higher apparent viscosity (Table I).

As previously reported (Arrese et al., 1991), influence

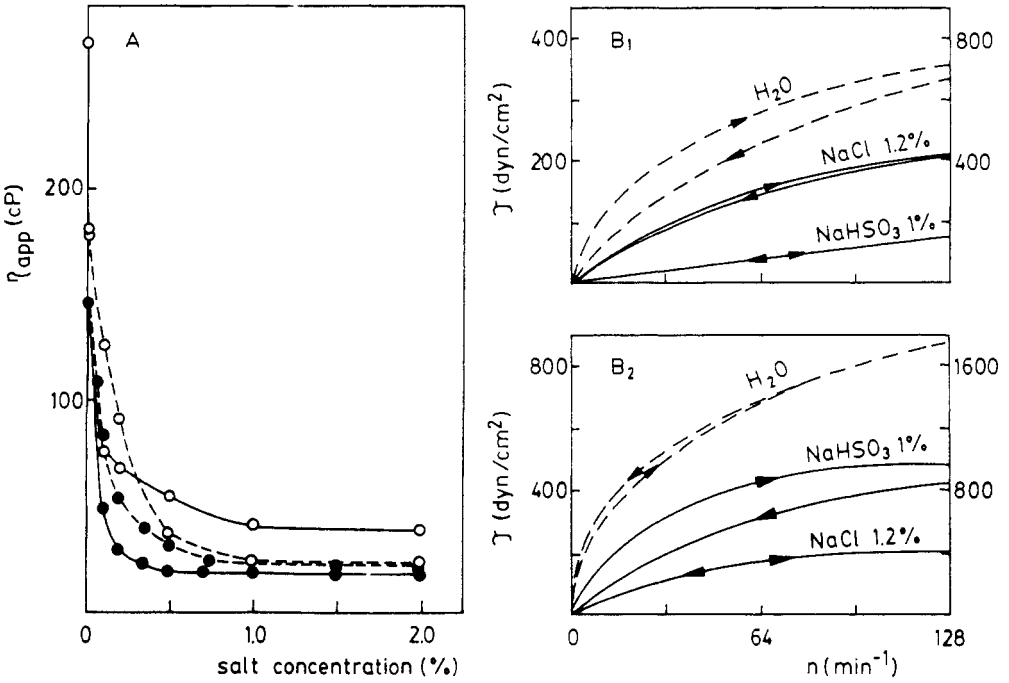

Figure 5. Effect of Na_2SO_3 and NaCl addition on apparent viscosity (η_{app}) and flow properties of soy protein isolate dispersions. (A) Representative isolate of group I (●) and of II (○). (—) Na_2SO_3 addition; (- - -) NaCl addition. (B) Flow curves of protein dispersion in water, with added Cl^- or with added SO_3^{2-}. (B_1) Representative isolate of group I (sample 1). (B_2) Representative isolate of group II (sample 6). Flow curves without salt addition: (- - -) right ordinate.

of WIC on η_{app} is better expressed as the relationship between the imbibed water ratio (T/I) and η_{app}.

Data of η_{app} of all commercial isolates studied in our laboratory fell between curves A and B (Arrese et al., 1991; Sorgentini et al., 1991; present study) (Figure 4). A theoretical curve (curve C) was calculated from the experimental points of all those samples. The shape of this curve is in accordance with the equation $\eta_f(T/I)^{-n}$. Samples with high WIC gave η_{app} values which fell in a region between curve A and the master curve C (first region). η_{app} values of those samples with lower WIC fell between curves B and C (second region). The protein concentration required to reach a specified value of T/I ratio is lower in samples of the first region, with high WIC, than in these samples with low WIC, in the second region (see vertical tracing on Figure 4 for T/I ratio = 2; sample on curve A, 3.9 mg/mL, and on curve B, 8.8 mg/mL).

For the same T/I ratio, those protein dispersions with higher protein content with show higher η_{app}. From the analysis of the behavior at equal protein concentrations, it can be seen that those samples with higher T/I will give lower η_{app} values (see Figure 4 points of isoconcentration). So, both variables have to be considered when η_{app} values of protein dispersions are involved.

Environmental Effect on Viscosity. The formation of aggregated products depends largely on the electrostatic atmosphere surrounding the protein molecule (Damodaran and Kinsella, 1982; Iwabuchi et al., 1991). A change in the electrostatic environment can be achieved by adding neutral electrolytes (Tanford, 1968).

The effect of sodium sulfite and sodium chloride on viscosity of protein dispersions at different concentrations and at pH 7 was studied. Figure 5 shows the results obtained from dispersions at 8% in a representative sample of each group. It can be observed that the addition of both ionic species (<0.5%) decreases the apparent viscosity, even at very low concentrations.

It was clearly observed, in previous work, that chloride as well as sulfite produces decreases in WIC (Wagner and Añón, 1990). The reduced WIC can be mainly attributed to the shielding effect of NaCl ion pairs, which prevent water molecules from interacting with protein molecules (Yao et al., 1988).

As WIC determines viscous behavior, these ions are also expected to influence viscosity.

The maximum effect is reached at 1% salt concentration ($\mu Cl^- = 0.171$, $\mu SO_3^{2-} = 0.174$, pK_{a2} $Na_2SO_3 = 6.91$; *CRC Handbook of Chemistry and Physics*, 1976) in every case (Figure 5A). The behavior, however, is not the same in all samples. In group I, the sulfite effect is higher than the chloride effect, although it tends to equalize at high concentrations. In group II, the chloride effect is higher than the sulfite effect even at high concentrations. The observed differences between both groups of isolates may be due to differences in structures, since denatured proteins form structures by interparticle hydrophobic interactions, while intermolecular interactions are mainly observed in native proteins.

In group III isolates there is not a net tendency because they have already been treated with reducing agents and calcium ion. Nonetheless, Cl^- as well as SO_3^{2-} diminishes viscosity.

Figure 5B shows the effect of the addition of chloride and sulfite at a concentration with maximum effect on both native and denatured samples of the second group (panels B_1 and B_2, respectively). The effect of Cl^- addition to denatured samples ($\Delta H = 0$) is higher than that on the partially denatured ones ($\Delta H > 0$), because it leads to the formation of interparticle aggregates since the hydrophobic groups or areas are exposed.

Addition of Cl^- ion to native samples only stabilizes the quaternary structure. This decreases intermolecular repulsive forces, resulting in less swollen particles (Hermansson, 1975; Shen, 1976). The decrease in viscosity in native samples could be explained by a loss of the capacity of water retention, while in denatured samples it would be related to the formation of more compact aggregates, unable to retain water.

At very low concentration, the disulfide bond cleaving agent sodium sulfite reduces the η_{app} values in inverse

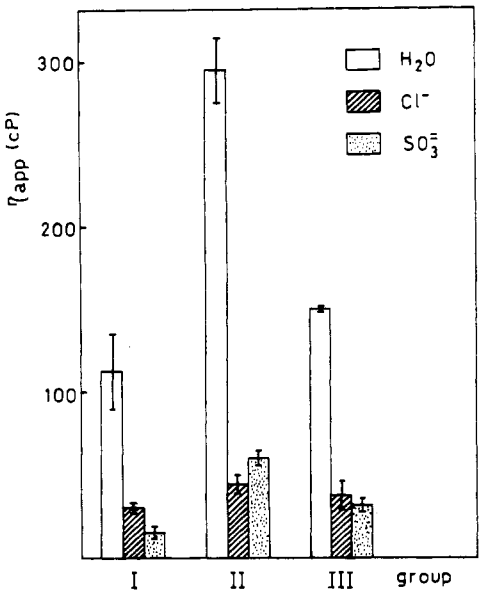

Figure 6. Effect of added Cl^- or SO_3^{2-} on apparent viscosity (η_{app}) of different groups of isolates. Isolate concentration in the protein dispersion was 10% (w/w).

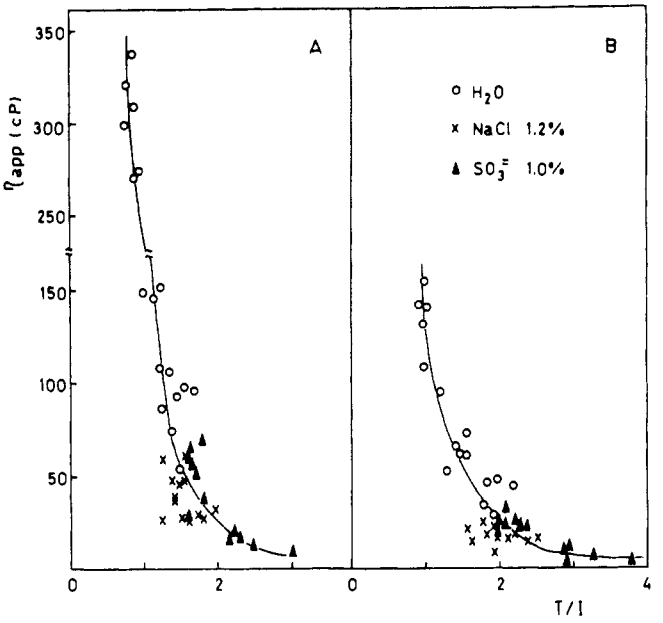

Figure 7. Changes in the relationship of apparent viscosity and T/I ratio by chloride or sulfite addition. Isolate concentrations were (A) 10 and (B) 8% (w/w). (○) In water; (×) with added Cl^- 1.2%; (▲) with added SO_3^{2-} 1%.

proportion: it has a higher effect on native samples because disulfide bridges are very important to mantain their structures. Denatured samples are less affected because such bridges would not be so important to stabilize aggregates capable of water absorption.

Although there are differences in the magnitude of Cl^- and SO_3^{2-} effects, the general tendency in all samples is an appreciable viscosity decrease due to the addition of such ions (Figure 6). This figure shows that, in most samples, sulfite produces a higher viscosity decrease than chloride. The isolates of group II, as it was already mentioned, show an inverse effect, while those of group III have an effect similar to that observed in group I; they are samples altered by other treatments (calcium ion, reducing agents, heating). If Cl^- and SO_3^{2-} effects influence WIC, the viscosity observed at increasing values of T/I will decrease. As shown in Figure 7, all samples follow the previously found correlation, so it can be reaffirmed that viscosity depends not only on the concentration of the protein isolate but also on WIC.

A wide variety of viscosities can be obtained from the variation of sample concentration and/or the addition of chloride or sulfite. Together with the viscosity, the area of hysteresis loops in the suspending systems decreased with increasing salt concentration (data not shown). These phenomena can be explained by the stabilization of the structures at higher ionic strength (Catsimpoolas and Meyer, 1970; Hermansson, 1978; Umeya et al., 1980).

These results indicate that the viscosity of a protein solution may be manipulated by shearing and chemical or physical modifications to create desirable conditions for further processing of proteins.

Effect of Thermal Treatment on Viscosity. To study the gelation capacity, some η_{app} measurements were carried out on 8% dispersions. This concentration was chosen to measure the viscosity increase under thermal treatment. To obtain information about rheological behavior, this study was performed on a Haake viscometer and not on a Brookfield texturometer, which is used in most gelation work, to compare viscosity values before and after heating.

Figure 8 shows the behavior of a representative sample of each group.

The measurements of apparent viscosity at 128 rpm show that the protein structure not only is capable of producing viscous dispersions but is also resistant to the shearing effect, which reflects its stability.

Heating produced a viscosity increase in all samples.

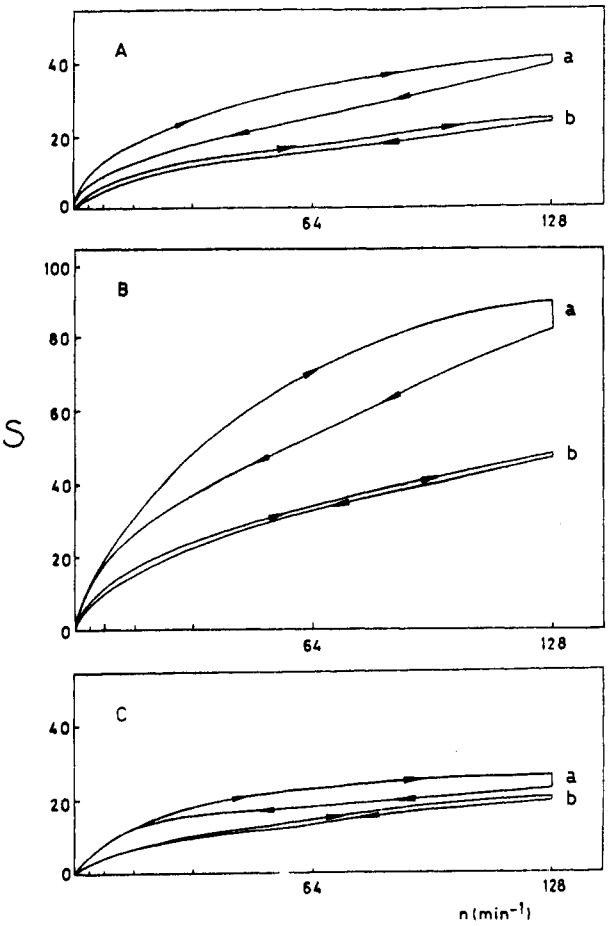

Figure 8. Thermal treatment effect on flow curves of representative isolates of groups I (A), II (B) and III (C) (samples 4, 6, and 17, respectively). Isolate dispersion 8% (w/w); thermal treatment, 80 °C for 30 min and 4 °C overnight. (a) Heated samples; (b) unheated samples. S, scale grade value; n, rotor speed (rpm).

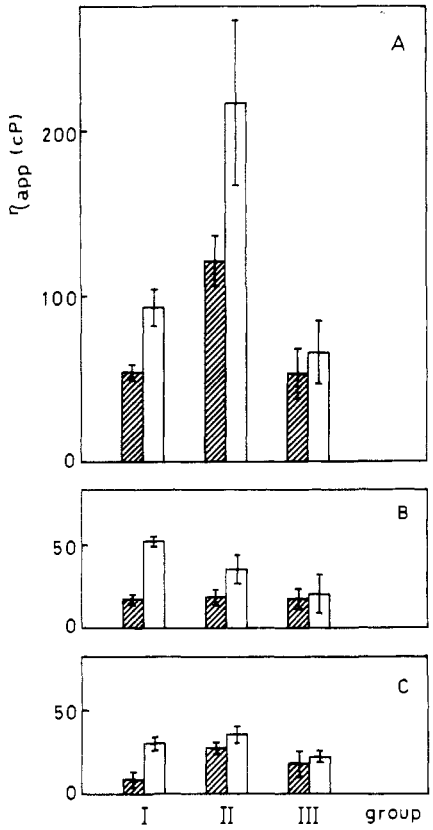

Figure 9. Thermal treatment effect on apparent viscosity (η_{app}) of 8% isolate dispersions. (A) In water; (B) with added Cl^- 1.2%; (C) with added SO_3^{2-} 1%. (Slashed bars) Unheated; (open bars) heated. Rate of shear was 128 rpm.

This suggests the existence, in all of them, of reactive protein species which due to heating are able to interact and form a matrix even in those samples with denatured proteins (groups II and III; Figure 9A).

The samples with a greater increase of their viscosity by thermal effect are those with partially denatured proteins (group I) and the denatured ones of group II. Those of group III have a smaller η_{app} increase on heating.

The capacity to form gels is directly related to availability, especially in the soluble fraction, of β subunits of 7S protein and basic polypeptides of 11S protein; that capacity decreases as the free SH groups increase. Most samples of group III have high contents of SH groups, very low solubility (Table I), and low contents of β subunits and basic polypeptides in the soluble fraction (Arrese et al., 1991). This was due to the fact that, during processing, they were not only denatured but also submitted to treatments which led to the reduction of S–S bridges and to the formation of insoluble aggregates that would not be available to form new structures.

The differences in η_{app} (measured at 128 rpm) between the first and the second groups suggest the possibility of a greater capacity to form new structures in samples already denatured (group II). Nevertheless, samples with native proteins might be expected to show outstanding changes on heating.

This apparent contradiction is resolved if η_{app} values are analyzed at low speed (Figures 9 and 10). Figure 10 shows that group I samples at 16 rpm give η_{app} values closer to those of group II. It also shows that the lower the rotor speed is, the closer the values are.

Measurements at low rotation speed, such as those carried out on a Brookfield viscometer (rpm <5), suggest gel hardness and not stability.

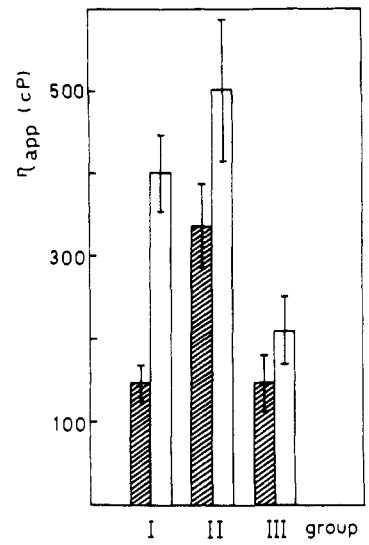

Figure 10. Thermal treatment effect on apparent viscosity (η_{app}) of 8% isolate dispersions at low rate of shear (16 rpm). (Slashed bars) Unheated; (open bars) heated.

η_{app} values increase on heating even in the presence of Cl^- and SO_3^{2-} (Figure 9B,C), especially in the samples of groups I and II. This would indicate that, under such conditions, there are still possibilities of new protein–protein interactions. Nevertheless, the attained values are much smaller than those obtained in water solutions. This shows, once again, the importance of hydrophobic interactions and S–S bridges in forming these structures.

CONCLUSIONS

Knowledge of the viscosity of protein dispersions is of practical significance in relation to processing and new product development. The results of this study indicate that the rheological properties of protein dispersions of commercial soy isolates are highly sensitive to both variables: moisture content (expressed as T/I ratio, determined by the WIC) and protein concentration, which are interdependent. The transition from a quasi-Newtonian to a pseudoplastic behavior, as protein concentration increases, became evident in an abrupt change to steeper slopes of the curves obtained in the double-logarithmic plots of η_{app} vs isolate concentration.

The different pseudoplastic behaviors reflect differences in the degree of denaturation and aggregation and have been taken as a criterion to divide commercial isolates into three groups. These groups, emerging from different flow properties, are in agreement with those groups defined according to the treatment undergone by the proteins during processing (Materials and Methods) or on the basis of functional and structural properties and ion content (Table I).

NaCl and Na_2SO_3 additions affect the WIC and, in consequence, the viscosity. η_{app} values decrease by salt effect and show significant differences according to the degree of denaturation of the isolates, which determines the type of aggregates formed. Again, isolates can be clustered in groups similar to those previously defined.

There is an increase of η_{app} with thermal treatment in all isolates. This increase, observed even in those dispersions with decreased viscosity due to addition of chloride or sulfite, could be ascribed to the formation of new structures and to the importance of hydrophobic interactions and disulfide bridges on those interactions.

This work indicates that the chemical or physical modifications of proteins to create specifically desirable

properties for further applications could be detected by studying the rheological behavior of protein dispersions.

Although these data provide a means for the study of conformational changes in protein molecules and in their state of aggregation, complementary analyses, such as determination of how these changes affect the particle size distribution, are needed.

LITERATURE CITED

AACC. *Approved methods of analysis*; American Association of Cereal Chemists: St. Paul, MN, 1983; Vol. II, Method 46-23.

Arntfield, S. D.; Murray, E. D.; Ismond, M. A. H. Influence of salts on the microstructural and rheological properties of heat-induced protein networks from ovalbumin and vicilin. *J. Agric. Food Chem.* **1990**, *38*, 1335–1343.

Arrese, E. L.; Sorgentini, D. A.; Wagner, J. R.; Añón, M. C. Electrophoretic, solubility, and functional properties of commercial soy protein isolates. *J. Agric. Food Chem.* **1991**, *39*, 1029–1032.

Beveridge, T.; Jones, L.; Tung, M. A. Progel and gel formation and reversibility of gelation of whey, soybean, and albumen protein gels. *J. Agric. Food Chem.* **1984**, *32*, 307–313.

Catsimpoolas, N.; Meyer, E. W. Gelation phenomena of soybean globulin. I—Protein-protein interactions. *Cereal Chem.* **1970**, *47*, 559–569.

Circle, S. J.; Smith, A. K. Functional properties of commercial edible soybean products. In *Seed proteins*; Inglett, G., Ed.; Avi: Westport, CT, 1972; pp 242–254.

Circle, S. J.; Meyer, E. W.; Whitney, R. W. Rheology of soy protein dispersions, effect of heat and other factors on gelation. *Cereal Chem.* **1964**, *41*, 157–172.

CRC Handbook of Chemistry and Physics, 56th ed.; Weast, R. C., Ed.; CRC Press: Cleveland, OH, 1976.

Damodaran, S.; Kinsella, J. E. Effects of ions on protein conformation and functionality. In *Food protein deterioration. Mechanisms and functionality*; Cherry, J. P., Ed.; ACS Symposium Series 206; American Chemical Society: Washington, DC, 1982; pp 327–357.

Dosaka, S.; Kawamura, S.; Izutsu, T.; Honda, Y.; Taneya, S. Changes in rheological and chemical properties of soy protein dope solution through increase of aging time. *Agric. Biol. Chem.* **1979**, *43*, 809–814.

Frisch, H. L.; Shima, R. The viscosity of colloidal suspensions and macromolecular solutions. In *Rheology. Theory and applications*; Eirich, F. R., Ed.; Academic Press: New York, 1956; Vol. I, pp 525–613.

Hermansson, A. M. Functional properties of proteins for foods. Flow properties. *J. Texture Stud.* **1975**, *5*, 425–439.

Hermansson, A. M. Physico-chemical aspects of soy proteins structure formation. *J. Texture Stud.* **1978**, *9*, 33–58.

Hermansson, A. M.; Akesson, C. Functional properties of added proteins correlated with properties of meat systems. 2. Effect of salt on water binding properties of model meat systems. *J. Food Sci.* **1975**, *40*, 603–610.

Holdsworth, S. D. Applicability of rheological models to the interpretation of flow and processing behavior of fluid food products. *J. Texture Stud.* **1971**, *2*, 393–418.

Holmes, A. B.; Gilbert, S. L.; Rha, C. K. Rheological properties of cranberry cell wall material. *J. Food Sci.* **1977**, *42*, 29–34.

Iwabuchi, S.; Watanabe, H.; Yamauchi, F. Observations on the dissociation of β-conglycinin into subunits by heat treatment. *J. Agric. Food Chem.* **1991**, *39*, 34–40.

Kinsella, J. E. Functional properties of proteins in foods: A survey. *Crit. Rev. Food Sci. Nutr.* **1976**, *7*, 219–280.

Lee, C. H.; Rha, C. K. Thickening of soybean protein suspensions with calcium. *J. Texture Stud.* **1977**, *7*, 441–449.

Lee, C. H.; Rha, C. K. Rheological properties of proteins in solution. In *Food texture and rheology*; Sherman, P., Ed.; Academic Press: New York, 1979; pp 245–263.

Lopez de Ogara, M. C.; Bercovich, F.; Pilosof, A. M. R.; Bartholomai, G. Denaturation of soybean proteins related to functionality and performance in a meat system. *J. Food Technol.* **1986**, *21*, 279–287.

Mohri, M.; Matsushita, S. Improvement of water absorption of soybean protein by treatment with bromelain. *J. Agric. Food Chem.* **1984**, *32*, 486–490.

Mossé, J. Nitrogen to protein conversion factor for ten cereals and six legumes or oilseeds. A reappraisal of its definition and determination. Variation according to species and to seed protein content. *J. Agric. Food Chem.* **1990**, *38*, 18–24.

Mulvihill, D. M.; Kinsella, J. E. Gelation of beta-lactoglobulin: Effects of sodium chloride and calcium chloride on the rheological and structural properties of gels. *J. Food Sci.* **1988**, *53*, 231–239.

Nakamura, B.; Sugiyama, M.; Sato, Y. Factors contributing to heat-induced aggregation of ovalbumin. *Agric. Biol. Chem.* **1978**, *42*, 819–824.

Ohren, J. A. Process and product characteristics for soya concentrates and isolates. *J. Am. Oil Chem. Soc.* **1981**, *58*, 333–335.

Pradipasena, P.; Rha, C. K. Pseudoplastic and rheopectic properties of globular proteins. *J. Texture Stud.* **1978**, *8*, 311–325.

Rakosky, J. Soy products for the meat industry. *J. Agric. Food Chem.* **1970**, *18*, 1005–1009.

Rha, C. K.; Pradipasena, P. Viscosity of Proteins. In *Functional properties of food macromolecules*; Mitchell, J. R., Ledward, D. A., Eds.; Elsevier Applied Science Publishers: London, 1986; Chapter 2, pp 79–120.

Saio, K.; Koyama, E.; Watanabe, T. Protein-calcium-phytic acid relationships in soybean. Part II. Effects of phytic acid on combination of calcium with soybean meal protein. *Agric. Biol. Chem.* **1968**, *32*, 448–452.

Shen, J. L. Solubility profile, intrinsic viscosity, and optical rotation studies of acid precipitated soy protein and of commercial soy isolate. *J. Agric. Food Chem.* **1976**, *24*, 784–788.

Sorgentini, D. A.; Wagner, J. R.; Arrese, E. L.; Añón, M. C. Water imbibing capacity of soy protein isolates: influence of protein denaturation. *J. Agric. Food Chem.* **1991**, *39*, 1386–1391.

Tanford, C. Protein denaturation. Part A. *Adv. Protein Chem.* **1968**, *23*, 121–282.

Torgensen, H.; Toledo, R. T. Physical properties of proteins preparations related to their functional characteristics in comminuted meat systems. *J. Food Sci.* **1977**, *42*, 1615–1620.

Umeya, J.; Yamauchi, F.; Shibasaki, K. Hardening and softening properties of soybean protein-water suspending systems. *Agric. Biol. Chem.* **1980**, *44*, 1321–1326.

Urbanski, G. E.; Wei, L. S.; Nelson, A. I.; Steinberg, M. P. Flow characteristics of soybean constituents controlled by ratio of total to imbibed water. *J. Food Sci.* **1983**, *48*, 691–694.

Varunsatian, S.; Watanabe, K.; Hayakawa, S.; Nakamura, R. Effects of Ca^{2+}, Mg^{2+}, and Na^{+} on heat aggregation of whey protein concentrates. *J. Food Sci.* **1983**, *48*, 42–46.

Wagner, J. R.; Añón, M. C. Influence of denaturation degree, hydrophobicity and sulfhydryl content of solubility and water absorbing capacity of soy protein isolates. *J. Food Sci.* **1990**, *50*, 765–770.

Wu, Y. V.; Inglett, G. E. Denaturation of plant proteins related to functionality and food applications. A review. *J. Food Sci.* **1974**, *38*, 216–225.

Yao, J. J.; Wei, L. S.; Steinberg, M. P. Water-imbibing capacity and rheological properties of isolated soy proteins. *J. Food Sci.* **1988**, *53*, 464–467.

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Registry No. NaCl, 7647-14-5; Na_2SO_3, 7757-83-7; Ca, 7440-70-2.