Thermal and Electrophoretic Behavior, Hydrophobicity, and Some Functional Properties of Acid-Treated Soy Isolates

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In the present work, changes in the structure and functional properties of soy protein isolates caused by mild acid treatment at room temperature were investigated. Different conditions (pH, time, neutralization procedure, and isolate concentration) of acid treatment and consequent salt increase were analyzed. The results obtained show that there is a selective denaturation of 11S protein, which conduces to higher surface hydrophobicity. The solubility of modified isolates decreases with extended storage period of the flour from which they were obtained and with higher isolate concentration during acid treatment. The high water imbibing capacity (WIC) of the resulted insoluble fraction does not produce significant changes in the WIC of the total isolate. The denaturation and dissociation of 11S protein lead to modified isolates with improved capacity to form and to stabilize foams without losing the gel formation capacity.

Keywords: *Soy protein; soy isolate; chemical modification; acid treatment; thermal behavior; functional properties*

INTRODUCTION

Soybean seeds contain the highest amount of protein in Leguminosae (30−50%) and have become an important protein source. Hence, there is a considerable amount of related research (Nielsen, 1985; Prakash et al., 1986; Kinsella et al., 1985).

Glycinin (11S) and β-conglycinin (7S) globulins are the major components of soy protein isolates. They must possess appropriate functional properties for food applications. These properties are influenced by the composition, structure, and conformation of ingredient proteins (Kinsella, 1979).

Isolation, commercial processing, and storage of soy protein isolates caused physical and chemical changes in the proteins (Shen, 1976; Ohren, 1981). Appropriate modifications could produce desired functional properties and improve their use as functional ingredients in the food industry. This may be achieved by chemical treatments. The acid treatment by its dissociating−denaturing action, and because of its easily applicability at industrial scale, is an interesting alternative. The acid-induced conformation changes of 7S and 11S proteins were due to dissociation of both proteins into subunits and unfolding of the polypeptide chains (Eldridge and Wolf, 1967; Koshiyama, 1972; Peng et al., 1984).

Improvement of functional properties was observed in mild acid treatments of soy (Matsudomi et al., 1985; N Guyen Thi Quynh et al., 1992), oat (Ma and Khanzada, 1987), and sunflower (Claughton and Pearce, 1989) protein isolates, in 11S soybean protein (Wagner and Guéguen, 1995), or in gluten (Ma et al., 1986; Popineau et al., 1988). Almost all these treatments include heating, which introduces additional modifications such as hydrolysis and deamidation.

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Acid medium without heating is a not exploited tool for modifying soy protein isolates for specific purposes. The aim of this study was to analyze if the simple action of acid medium, without any other treatment, was able to improve some functional properties of soy protein isolates obtained as usual in laboratory and industry. For this purpose, the relationship between changes in structural and functional properties of acid-modified soy proteins was studied. Acid treatment implies an increase of salt content whose effect was also studied.

MATERIALS AND METHODS

Native Soy Isolates. Native isolates A and B were prepared from defatted soybean flours (Sanbra S. A. Brazil) stored at room temperature for less than 1 month and more than 1 year, respectively. Flours were mixed with 10 times their weight of distilled water and adjusted at pH 8.0 with 2 N NaOH. The dispersions were gently stirred for 2 h at room temperature and then centrifuged at 5600 rpm (GSA-rotor, Sorvall RC 5B). The extracts were adjusted to pH 4.5 with 1 N HCl, kept 2 h at 4 °C, and centrifuged at 6500 rpm (Sorvall GSA-rotor) for 20 min at 4 °C. The precipitates were washed with water, resolubilized in water by neutralization at pH 8.0 with 1 N NaOH at room temperature, and lyophilized (Thermovac Industries Corp. freeze dryer).

Acid-Modified Soy Isolates. Aqueous dispersions (2−8% w/w) of native isolate (A or B) were adjusted to acid pH (1.0 to 3.5) with 1 N HCl and kept during 0−24 h at room temperature. The dispersions were neutralized with 1 N NaOH in one step (acid pH → pH 8) or in two steps [acid pH → pH 4.5 (2 h) → pH 8] and lyophilized.

Soluble and Insoluble Fractions. Aqueous dispersions of native and modified soy isolates (4% w/w) were centrifuged at 6500 rpm for 30 min (Sorvall GSA-rotor). Supernatant and precipitate (soluble and insoluble fractions) were separated, collected, and lyophilized.

Enriched 7S and Crude 11S Fractions. These fractions were prepared from defatted soy flour by the procedure of Thanh and Shibasaki (1976). The purity of 7S and 11S fractions (determined by SDS−PAGE) was approximately 70% and 90%, respectively.

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

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Chloride Content. A 10 g sample was carbonized with a Bunsen burner. The carbonized residue was suspended in hot distilled water and then filtered through Whatman no. 1 paper. On the filtrate, ion chloride content was determined using the Mohr method as described by Kolthoff and Sandell (1941), by titration with 0.1 N $AgNO_3$ solution using $KCrO_4$ as an indicator.

Solubility. The solubility of total native or modified isolates was expressed as the percentage (w/w) of soluble fraction obtained.

Protein Determination. Protein concentration was determined using the Biuret method (Gornall et al., 1949). The Lowry method (Lowry et al., 1951) was used as an alternative for diluted solutions.

Differential Scanning Calorimetry (DSC). The samples were analyzed in a DuPont Model 910 calorimeter attached to a Hewlett-Packard 7046B recorder. The areas under the endotherm curves were measured with a Morphomat 34 Zeiss image analyzer, and the corresponding enthalpies of thermal denaturation (ΔH in joules per gram of dry matter) were calculated. The specific areas for each peak of the endotherm (partial area per total milligram of dry sample) were also determined. Denaturation degree (%) was calculated as the specific area (corresponding to total endotherm or peak I or II) of treated isolate with respect to the same specific area of native isolate. All the assays were performed as previously described (Wagner and Añón, 1990) and repeated at least twice.

Surface Hydrophobicity (S_o). S_o was determined by the hydrophobicity fluorescence probe 1-anilino-8-naphthalenesulfonate (ANS). Measurements were performed according to Kato and Nakai (1980). Protein dispersions (1 mg/mL) in 0.01 M phosphate buffer (pH 7.0) were stirred 2 h at 20 °C and centrifuged at 6500 rpm (Sorvall, SS34-rotor) for 20 min. Each supernatant was serially diluted with the same buffer with and without 0.5 M NaCl to obtain protein concentrations ranging from 0.1 to 0.0005 mg/mL. Then 40 μL of ANS (8.0 mM in 0.1 M phosphate buffer, pH 7.0, solution) was added to 2 mL of sample. Fluorescence intensity (FI) was measured with a Perkin-Elmer 2000 fluorescence spectrometer, at wavelengths of 365 nm (excitation) and 484 nm (emission). The initial slope (S_o) of FI versus protein concentration plot (calculated by linear regression analysis) was used as an index of the protein hydrophobicity.

Electrophoresis (SDS–PAGE). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was carried out. Runs were performed in 5–15% polyacrylamide gradient gels in Bio-Rad mini-Protean electrophoresis equipment at a constant voltage (200 V). A continuous and dissociating buffer system, containing 0.375 M Tris-HCl, pH 8.8, and 0.1% SDS for the separating gel and 0.025 M Tris-HCl, 0.192 M Gly, and 0.1% SDS, pH 8.3, for the runs buffer, was used. Gel slabs were fixed and stained simultaneously in a solution of methanol, acetic acid, and water (5:5:2) and 0.1% Coomassie Brilliant Blue R-250. Molecular weights of the proteins were estimated by means of the MW-SDS-70L Sigma kit (α-lactalbumin, 14 200; trypsin inhibitor, 20 100; trypsinogen, 24 000; carbonic anhydrase, 29 000; glyceraldehyde-3-phosphate dehydrogenase, 36 000; ovalbumin, 45 000; albumin bovine, 66 000). Densitograms were obtained by means of a TLC Scanning CS-910 double-wavelength Shimadzu spectrodensitometer by scanning at 570 and 395 nm, for the sample and the reference, respectively. The areas under the densitometric curves were measured with a Morphomat 34 Zeiss image analyzer.

Water Imbibing Capacity (WIC). The WIC of soy protein isolates and their fractions 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 samples.

Gelling Formation. Aqueous dispersions (9–11% w/w) of native or modified soy protein isolates were heated at 100 °C for 15 min and then cooled overnight at 4 °C. The formation of self-supporting gel was examined by visual observation.

Foaming Properties. Foam formation was measured using a graduate glass column having a fritted glass disk (G4 type) at the bottom. N_2 gas was sparged at a flow rate of 180 mL/min through 30.6 mL of 0.5–10 mg/mL of total or soluble fractions of native or modified isolates in both water and 0.5 M NaCl solution, until a fixed volume of foam was reached (275 mL); the time to reach this volume (t_f) was registered. Determinations were performed by duplicate. The volume of the remaining aqueous phase at different times until the end of bubbling was recorded. Volumes of liquid incorporated to the foam were calculated, being V_{max} (maximum volume in mL), the volume at the end of bubbling. Foam density (D, dimensionless) was calculated as the ratio between volume of liquid incorporated into the foam and the corresponding foam volume reached. The rate of liquid incorporation to the foam (v_i, mL/min) was determined. Foaming capacity (FC) was measured as $FC = 275/(180t_f)$ (dimensionless).

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

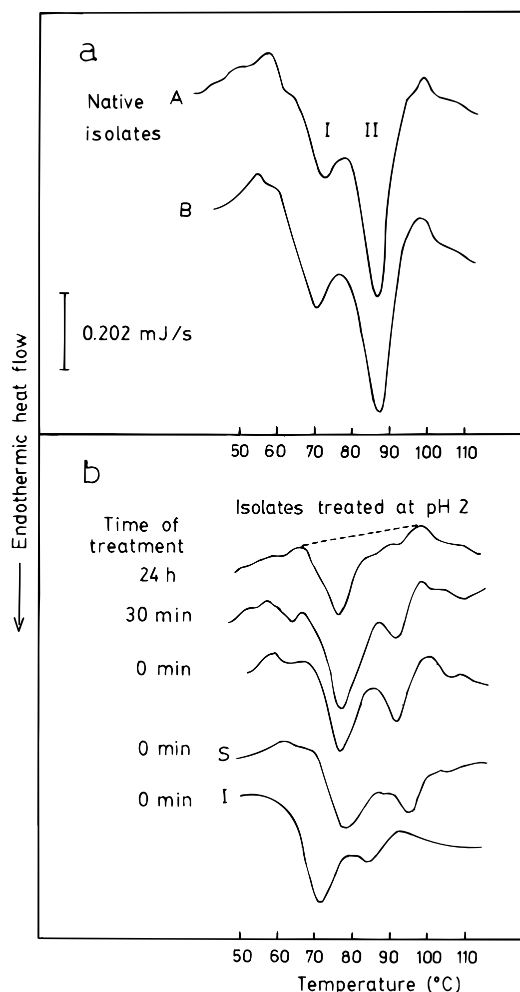

Figure 1. DSC thermograms of 16% (w/w) dispersions of native (a) or treated (b) isolates in distilled water. Heating rate, 10 °C/min. Peaks I and II correspond to thermal denaturation of 7S and 11S proteins, respectively. S and I correspond to soluble and insoluble fractions, respectively. DM (dry matter weight) was 2.90–3.10 mg.

RESULTS AND DISCUSSION

Native Soybean Isolate Characteristics. Figure 1a shows the DSC thermograms of two native isolates obtained in the laboratory from defatted soybean flour stored at 20 °C for less than 1 month (isolate A) and more than 1 year (isolate B). The DSC thermograms of such isolates did not exhibit differences in T_{max} nor in the areas of the characteristic peaks I and II which

Table 1. Solubility, WIC, and Denaturation Entalphy of Total Native and Treated Isolates and Their Fractions[a]

soy isolates	S (%, w/w)[c]	WIC (mL/g)[c] total isolate	WIC (mL/g)[c] soluble fraction	WIC (mL/g)[c] insoluble fraction	ΔH (J/g)[c] total isolate	ΔH (J/g)[c] soluble fraction	ΔH (J/g)[c] insoluble fraction
(a) native A	99.8	0.6	0.6		17.9	17.9	
(a) native B	90.7	4.5	4.2	4.6	17.6	17.6	17.1
(b) treated at pH 2[b]							
A	99.0	2.1	2.1		8.1	8.1	
B	71.5	4.0	1.1	11.1	7.1	6.4	7.2

[a] S, solubility; WIC, water imbibing capacity; ΔH, denaturation enthalpy from DSC thermograms. [b] Treatments were performed with 6% native isolate (A or B) concentration, for 60 min followed by fast neutralization (see Materials and Methods). [c] Each value is the result of at least two determinations.

correspond to the endothermic transitions of proteins 7S (β-conglycinin, $T_{max} = 70 \pm 1$ °C) and 11S (glycinin, $T_{max} = 86 \pm 1$ °C) (Hermansson, 1978; Wagner and Añón, 1990; Arrese et al., 1991). Although the storage did not modify the denaturation degree (equal ΔH), it did affect the protein aggregation state, which is reflected both by a solubility (% S) decrease and by an increase in the water imbibing capacity (WIC) (Table 1a). The aqueous extracts of such stored flours would contain proteins in an incipient aggregation state, which would tend to become partially insoluble during the subsequent preparation processes of the isolate [isoelectric precipitation (pH 4.5), neutralization, and drying]. The thermograms of soluble and insoluble fractions of isolate B were similar to that of the total isolate, and their ΔH values did not show significant differences (Table 1a), which indicates that the insoluble fraction would be formed by aggregates of nondenatured proteins 7S and 11S. The presence of aggregates both in the soluble fraction and in the insoluble one would explain the higher WIC of isolate B (Table 1a).

Effect of Acid Treatment (pH 2.0). In order to study the effect of the acid medium on isolates A and B, a preliminary test at pH 2 was performed. Table 1b shows that, for isolate A, this treatment did not produce any loss of solubility, though there was a protein denaturation of 54.7% [$(\Delta H_{treated}/\Delta H_{native}) \times 100$; $\Delta H_{treated} = 8.1$ J/g] and a slight increase of the WIC, which would indicate the existence of soluble aggregates constituted by denatured proteins. On the contrary, the treatment of isolate B at pH 2 led to a 21% decrease in the solubility. This could be ascribed to the previous existence of soluble aggregates (deduced by the WIC of the soluble fraction in the native isolate) which become insoluble by acid medium action. The resultant insoluble fraction possessed high water imbibing capacity, but the WIC of the total isolate did not change since at the same time the WIC of the soluble fraction decreased.

Table 1b also shows that the protein denaturation of isolate B by treatment at pH 2 was 59.6%. The insoluble fraction gave a ΔH equivalent to that of the total isolate, which would indicate that there is an equal distribution of denatured proteins and not a selective aggregation of denatured proteins leading to their insolubilization. Therefore, the protein aggregation mechanism in acid medium is not governed by the denaturation state as observed in thermally-treated soy isolates (Arrese et al., 1991; Sorgentini et al., 1995). In the latter, the insoluble fraction was mainly formed by denatured proteins.

The WIC and solubility were more affected in isolate B than in A by treatment at pH 2, so we selected isolate B to analyze the effect of several variables in treatments at that pH value, with the aim of studying later the effect of acid medium at various pH values under conditions where the changes can be detected more clearly.

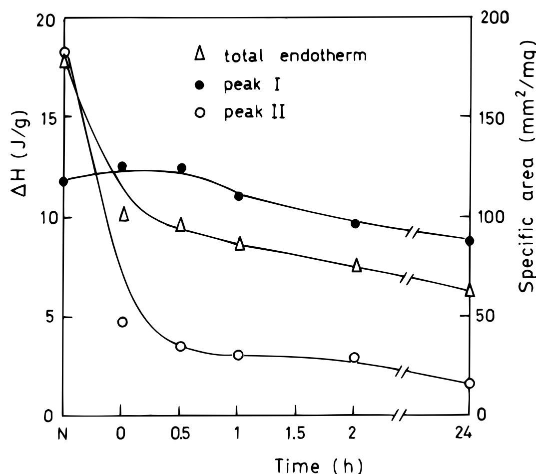

Figure 2. Effect of time of acid treatment (pH 2.0, 6% isolate concentration) on thermal denaturation enthalphy and specific areas corresponding to DSC thermograms. N, native isolate.

Effect of Time, Protein Concentration, and Neutralization Procedure on Acid Treatment. By maintaining aqueous dispersions of isolate B at 6% w/w and pH 2 during several periods, we obtained modified isolates, the DSC thermograms of which are shown in Figure 1b. It can be observed that, as the exposure time at pH 2 increases, the peak II area gradually decreases and practically disappears after a treatment of 24 h. On the contrary, peak I is not mostly affected even after treatment for 24 h. Besides, the thermograms of treated isolates show a shift in the T_{max} of peaks I and II toward higher values than those of native isolates (Figure 1a). Such a thermal stability increase in proteins of treated isolates is due to the higher NaCl content (as a result of adjusting the given pH with HCl with subsequent NaOH neutralization) (Hermanson, 1978; Damodaran and Kinsella, 1982a; Sorgentini et al., 1991). When separating the soluble and insoluble fractions of such isolates, it was observed that the T_{max} increase by the saline effect is much stronger in the soluble fraction which retains most of the salt (Figure 1b). In Figure 2, we plotted ΔH values and specific areas of peaks I and II as a function of acid treatment time. It can be observed that protein 11S experiences a denaturation of 70% in an instantaneous treatment at pH 2, while protein 7S begins to be affected only after 60 min. This indicates a higher sensitivity of glycinin to the denaturing action of the acid medium. From the results obtained, it can be inferred that, by controlling the acid treatment time, a selective denaturation of protein 11S can be reached without affecting protein 7S considerably. We observed, on the contrary, that the duration of the acid treatment does not have a significant influence on the solubility (% $S = 76.1 \pm 5.0$ between 0 and 24 h) nor on the WIC of the treated isolate (Table 2).

Table 2. Influence of the Neutralization Procedure after Acid Treatment on Solubility and WIC of Treated Samples[a]

acid treatment at pH 2.0[b]	S (%, w/w)	WIC (mL/g) total isolate	WIC (mL/g) insoluble fraction	WIC (mL/g) soluble fraction
(a) fast neutralization	76.1 ± 5.0	4.3 ± 0.3	11.3 ± 0.8	∼1
(b) neutralization for 120 min at p*I*	66.1 ± 2.7	4.6 ± 0.5	12.8 ± 0.5	∼1

[a] Values shown are the means ± standard deviations of a minimum of two determinations for each treatment period (0, 15, 30, 60, 120, and 240 min and 24 h). *S* and WIC as in Table 1. [b] Treatments were performed at 6% native isolate B concentration for 0–24 h.

Table 3. Influence of Isolate Concentration during Acid Treatment[a] on Solubility and WIC of Treated Isolate

isolate concentration (%, w/w)	S (%, w/w)	WIC (mL/g) total isolate	WIC (mL/g) insoluble fraction	WIC (mL/g) soluble fraction
1–2	90.0	2.5	6.9	2.4
4	72.0	3.3	9.5	1.3
6	71.5	3.9	10.2	1.2
8	68.0	4.0	11.0	1.0

[a] Treatments were performed with variable concentrations of native isolate B at pH 2.0 for 60 min, followed by fast neutralization. Values are the means of at least two determinations. *S* and WIC as in Table 1.

The neutralization procedure does not influence the degree of protein denaturation reached (there are no changes of ΔH; results not shown) nor does it modify appreciably the WIC of the total isolate or those of its fractions (Table 2). However, the neutralization procedure does have influence on the isolate solubility. Actually, the solubility decrease is enhanced if the pH is kept 120 min at the p*I* value during neutralization. Keeping isolates at the isoelectric pH would favor a stronger insolubilization of preformed aggregates.

On the basis of the previous results, we established the treatment conditions for the subsequent tests: 60 min at acid pH followed by rapid neutralization.

When the protein concentration at which treatment at pH 2 was performed was varied from 1 to 8% w/w, no changes in the denaturation degree were observed ($\Delta H = 7.3 \pm 0.7$ J/g). In Table 3 it is observed that, when the treatment is performed at low isolate concentrations (1–2%), the solubility decrease is minimum. On the other hand, the increase of protein concentration during acid treatment leads to increasing loss of isolate solubility. The greater the isolate concentration, the greater the WIC of the insolubles obtained, but, as in previous cases, these conditions did not improve the WIC of the total isolate with respect to that of the untreated one (Table 3). Therefore, in order to study the effect of acid treatment on both isolates, it was decided to proceed with the conditions selected in the preliminary tests (6% w/w).

Effect of pH Variation on Acid Treatment. The effect of pH on the protein denaturation degree of treated isolates under the selected conditions (60 min, isolate concentration of 6% w/w, quick neutralization) is shown in Figure 3a. The decrease in the pH of the treatment causes a gradual increase in the denaturation of 11S which is reflected by the reduction in peak II in its corresponding thermograms; besides, the shifts in T_{max} in isolates treated at low pH values (pH <2) are more evident here. This was attributed only to the saline effect since, as can be observed in Figure 5a, such

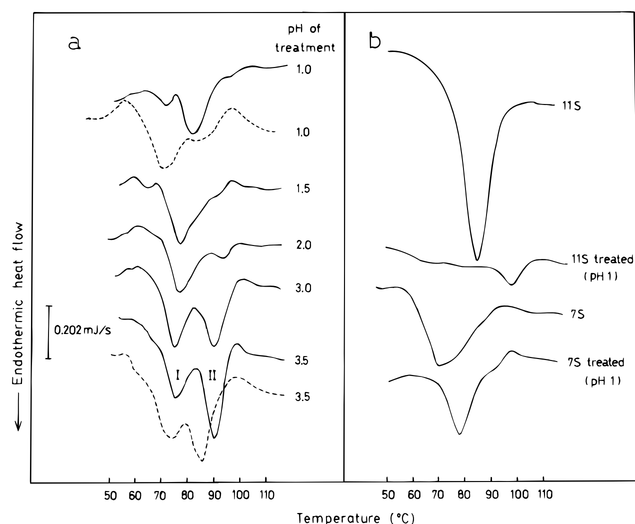

Figure 3. DSC thermograms of 16% (w/w) aqueous dispersions of (a) treated isolates and (b) native and acid-treated purified partially 7S and 11S proteins. (—) Without dialysis; (---) dialyzed against distilled water (3 times, 20 volumes each). DM (dry matter weight) was 2.70–3.00 mg.

a shift (in both peaks) correlates to the amount of NaCl (%, w/w) reached in the neutralization stage (Figure 5b). The DSC thermograms of treated isolates subsequently dialyzed (salt free) show that the T_{max} values of peaks I and II coincide with those of the native one (Figures 3a and 5a).

From the analysis of the effect of treatment at pH 1 on partially purified 7S and 11S proteins isolated, we were able to verify that acid pH produces a stronger denaturing action on protein 11S and that, in samples treated at low pH values, the remaining peak (pH 1.5, T_{max} = 76.5 °C; pH 1.0, T_{max} = 82.0 °C) corresponds to 7S (peak I in the thermogram of the native isolate) (Figure 3b). While this treatment did not modify substantially the denaturation degree of 7S (we observed only the characteristic shift of T_{max} = 78.5 °C), it did lead to an almost total denaturation of 11S. With these results, it can be inferred that the peak appearing with a T_{max} of 73.0 °C in the thermogram of the isolate treated at pH 1 (Figure 3a) can be attributed to the thermal transition of whey soy proteins. The DSC endotherm of denaturation of Kunitz trypsin inhibitor had a T_{max} = 60.0 °C (Anderson, 1992), and that of 2.8S soybean globulin fraction (which would correspond to whey soy proteins) had a T_{max} = 66.0 °C (Bikbov et al., 1986). In the thermogram of native isolates in Figure 1a, the shoulder observed with a T_{max} = 63 °C could be ascribed to these proteins. In the thermogram of isolate treated at pH 1.0, the peak with T_{max} = 73.0 °C may be whey soy proteins which are denatured at higher temperature owing to the presence of salts. At other treatment conditions (pH ≥2) and in native isolates, this peak would be overlapped by the thermal transition of the 7S protein. These results confirm that there are marked differences in the response of the various soybean proteins when undergoing acid-induced denaturation (Koshiyama, 1972).

The denaturation degree reached by isolates A and B after treatment in acid medium at several pH values is shown in Figure 4. In the region of lower pH values, the denaturing action is stronger on isolate B because its proteins were already sensitive owing to prolonged storage. Besides, at pH 3.5 there is already a denaturing effect (37.3%) on 11S of this isolate, which increases rapidly at pH 2.5 (87.2%) up to an almost total dena-

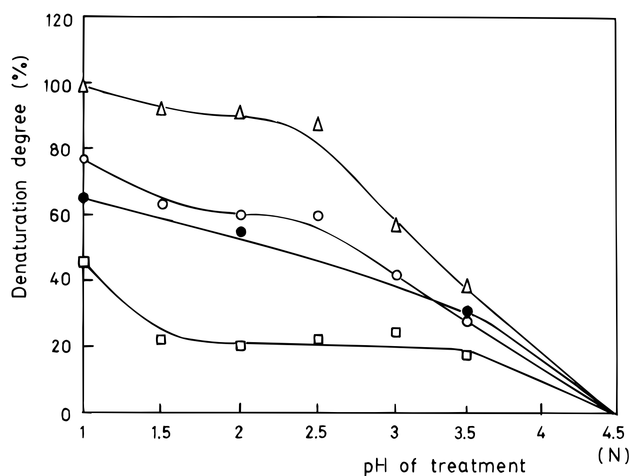

Figure 4. Denaturation degree as a function of pH of treatment. Percent denaturation degree was calculated from DSC thermograms, as described under Materials and Methods. (●, ○) Total endotherm of native (N) and treated isolates A and B, respectively; (□, △) specific areas of peaks I and II, respectively.

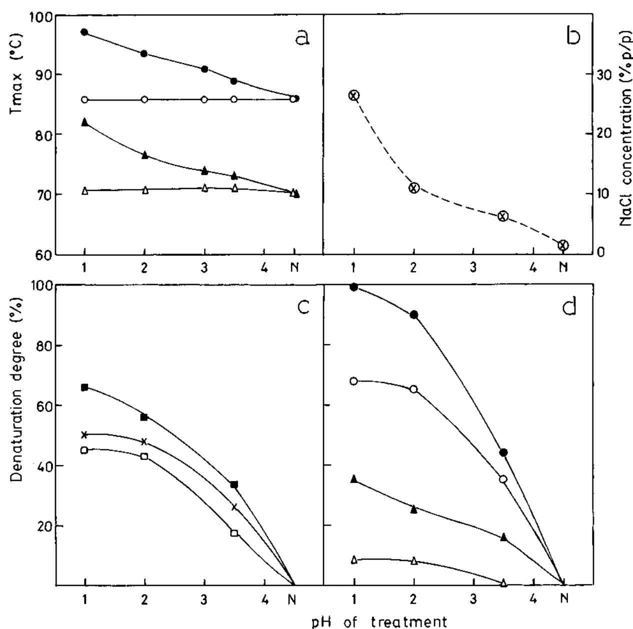

Figure 5. Effect of pH of acid treatment (6% w/w isolate concentration, 60 min) on DSC thermograms. Dialyzed (○, △, □) and nondialyzed (●, ▲, ■) isolates, respectively. (a) Transition temperature (T_{max}) of peaks I (●, ○) and II (▲, △); (b) NaCl concentration in isolates as a result of neutralization after acid treatment (⊗); (c) denaturation degree percentage of total area; ΔH calculated referred to dry matter weight (■, □) and corrected by recalculation of ΔH of (■) referred to dry protein weight (×); (d) denaturation degree percentage of 7S and 11S proteins [specific areas of peaks I (●, ○) and II (▲, △)]. N, native isolate.

turation at pH 1.0 (99%). In contrast, the denaturation degree of 7S is low (around 20% between pH 3.5 and 1.5) and increases only up to 45% at very acid pH values (pH 1).

The treated isolates have, as mentioned above, variable amounts of NaCl (Figure 5b). The presence of salts leads to two errors in the calculation of ΔH: one is due to the use of total dry matter values (with variable protein content) and the other one related to the influence of NaCl on protein thermal sensitivity. When the first was corrected by recalculating the ΔH with reference to dry protein weight (Figure 5c), the denaturing effect of the acid medium is still evident; however,

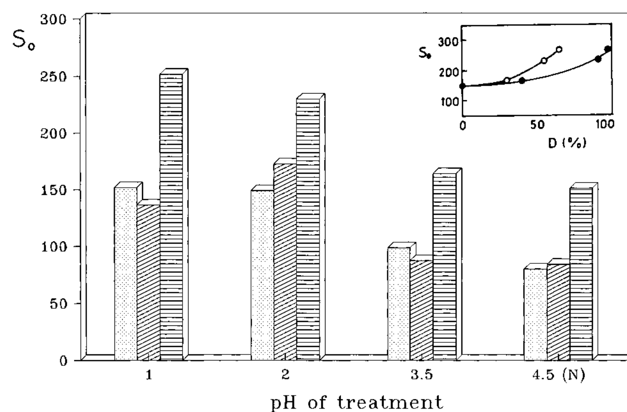

Figure 6. ANS surface hydrophobicity (S_0) of native (N) and modified isolates. Measured in 0.01 M phosphate buffer, pH 7: stippled and hatched bars refer to nondialyzed and dialyzed isolates, respectively. Measured in 0.01 M phosphate buffer, pH 7, with 0.5 M NaCl: horizontally striped bars refer to nondialyzed isolate. N, native isolate. (Inset) S_0 values as a function of denaturation degree (D%) of total protein (○) and 11S protein (●).

the difference between isolates treated at pH 1 and 2 now becomes smaller. When both error sources were eliminated by dialysis, the denaturation percentages, though lower, keep the same trend. This confirms that there is an irreversible denaturing effect of the acid medium. The differences in denaturation degree among dialyzed and nondialyzed samples are shown in Figure 5d but now in a separate analysis of the saline effect on peaks I and II. It is observed that the denaturing effect on protein 7S that can be attributed solely to the acid treatment is not greater than 10% even in samples treated at pH 1.

The denaturing effect of the acid medium is also reflected by changes in the surface hydrophobicity (S_0). It can be observed in Figure 6 that, as the pH decreases, there is an increase in surface hydrophobicity. The measurements of the latter are also affected by the presence of salts as a consequence of modifications in the hydrophobic interactions (Damodaran and Kinsella, 1982b). In order to decrease the impact of the variable contents of NaCl on the samples, we determined the hydrophobicity both at high NaCl concentrations (0.5 M) and in the absence of salts (dialyzed samples). Even when the S_0 values at 0.5 M NaCl were higher than those at standard conditions (because of stronger protein−ANS interactions), the evolution of hydrophobicity with pH keeps the same tendency. However, the dialyzed samples show a decrease of the S_0 reached at pH 1.0 in relation to the value obtained at pH 2.0. This would be due to aggregation of highly denatured 11S proteins caused by hydrophobic interactions in the cold dialysis process. There is a relationship between the denaturation degree induced by treatment in acid medium and the simultaneous increase of S_0. In this regard, an appreciable increase in the surface hydrophobicity requires a denaturation degree higher than 40% (inset, Figure 6). Owing to the observed increase of S_0, the modified isolates are expected to improve their capacity as surface agents (Wagner and Guéguen, 1995; Kim, 1985; Kato and Nakai, 1980).

When studying the solubility of isolates treated at pH 1, 2, and 3.5, isolate B showed solubility percentages of 62.0, 71.5, and 82.0, respectively. The lower solubilities found in the more acid zone would be due to greater protein aggregation caused by a gradual increase in the denaturation of 11S plus the aggregation induced by the

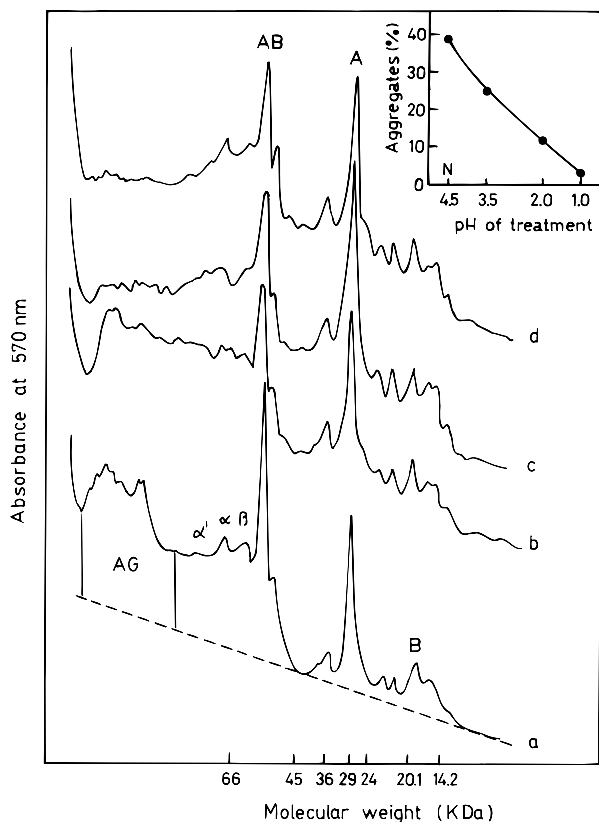

Figure 7. Densitometric scans of the electrophoretic patterns (SDS−PAGE): (a) native isolate B; (b, c, d) treated isolates at pH 3.5, 2.0, and 1.0, respectively. AG, 140−200 kDa aggregates; α′, α, *β*, 7S subunits; AB, A, B, 11S subunit, acid and basic 11S polypeptides, respectively. (Inset) aggregate percentage in each densitogram scan.

increase of the saline concentration. These results agree with those obtained from SDS−PAGE analysis of the soluble fraction of isolate B. Figure 7a shows the existence of a high percentage of high molecular mass (from 140 to 200 kDa) aggregates in the native isolate that decrease in the isolates modified at acid pH (Figure 7b,c,d). In the inset of Figure 7, it is observed that the high percentage of this fraction in the native isolate decreased gradually as the treatment was done at lower pH values, which indicates a passage of the soluble aggregates to the insoluble fraction and/or the formation of bigger aggregates, which are unable to enter the gel. The relative areas of the peaks of the subunits did not change considerably with acid treatments, and this would indicate, unlike what was observed in thermal or alkaline treatments, that there is no selective aggregation among subunits or dissociation of the intermediate subunit AB in 11S (Utsumi et al., 1984; Arrese et al., 1991; Sorgentini et al., 1995). On the contrary, when isolate A was treated in acid medium, the resultant modified isolates were totally soluble. According to these results, the proteins of isolates obtained from flours stored for extended periods would be more easily aggregated by treatment in acid medium. On the other hand, these treatments, even at extreme pH values, did not improve the WIC of isolates A and B.

Effect of pH of Acid Treatment on Foaming Properties. A number of workers have shown that solubility has an important influence on the foaming behavior of proteins (Hermansson et al., 1971; Wang and Kinsella, 1976). Proteins for foaming should be soluble in the aqueous phase; they should concentrate at the interphase, unfold to form cohesive layers around air bubbles, and possess enough viscosity and mechanical strength to prevent rupture and coalescence (Kinsella, 1979).

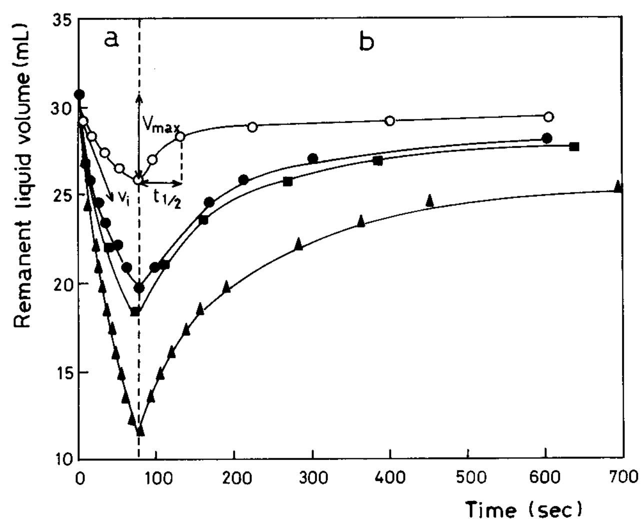

Figure 8. Remaining liquid volume as a function of time during foam formation (a) and foam destabilization (b). (---) End of N_2 bubbling. Assays performed with aqueous dispersions (2 mg/mL) of native (○) and treated isolates at pH 3.5 (●), 2.0 (■), and 1.0 (▲).

As the foaming capacity depends on the soluble fraction, it was therefore studied on totally soluble modified isolates which were obtained from isolate A. Figure 8 shows the profiles obtained when determining the foaming properties of aqueous dispersions (2 mg/mL) of native isolates and of those treated at several pH values. In the figure, the meaning of the parameters v_i, V_{max}, and $t_{1/2}$ is indicated. The first part (a) of the curves corresponds to the incorporation of liquid into the foam during foam formation, whereas the second part (b) represents the drainage of liquid from the foam (a measure of foam instability after the end of bubbling). It can be observed that the lower the pH of acid treatment the more the modified isolates favors foam formation (greater v_i and V_{max}, and hence greater foam density when reaching its maximum volume).

For all isolates tested, the foaming capacity (FC) values were 0.90 ± 0.05, which means a high and equivalent retention of N_2 during foam formation by bubbling. Therefore, it is not possible to use FC as a parameter to evaluate the foaming behavior of these isolates. It was found that differences were not in the foam volume but in the quality of it, the latter being determined by the amount of incorporated liquid and by the draining rate of it.

Figure 9a shows that v_i increases not only for decreasing pH of treatment but also for increasing concentrations of native and modified isolates in the assay. However, at the same time as the isolate concentrations increase, the differences of v_i between native and modified isolates become smaller.

In Figure 9b, it is observed that in foaming tests with aqueous dispersions having 2 mg of isolate/mL, the foam stability is much higher in those isolates modified at lower pH, which is evidenced by a decrease in K (the combined result of higher $t_{1/2}$ and V_{max}). An increase in isolate concentration in the dispersions tends to equalize the foam stability ($K = 0.01 \pm 0.002$ at 10 mg/mL). The values of K and v_i obtained with aqueous dispersions of native isolate at high concentration can also be reached by aqueous dispersions of modified isolate at low concentrations.

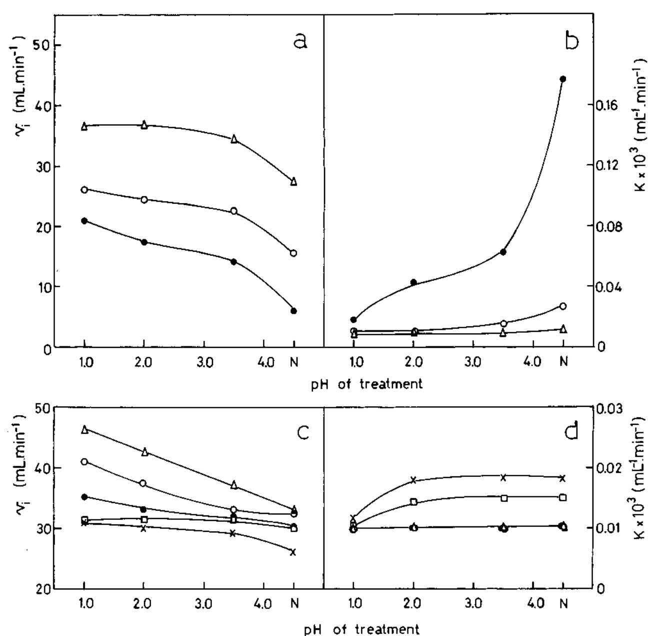

Figure 9. Rate of liquid incorporation into the foam (v_i) and specific rate constant of drainage (K) as a function of pH of treatment. Foaming assays were performed in aqueous dispersions without (a, b) or with (c, d) 0.5 M NaCl dispersions of native (N) and acid-treated isolates at 0.5 (×), 1.0 (□), 2.0 (●), 5.0 (○), and 10.0 (△) mg/mL.

As seen previously, the isolates treated at acid pH have variable NaCl content (Figure 5b). This made us aware of the fact that the comparison of foaming properties involves the contrast of solutions with the same isolate concentration but with different saline and protein content. With regard to the first, it is observed that the isolate treated at pH 1 possesses the best foaming properties (Figure 9a,b), but we cannot discern whether this is due to protein modifications or to the higher saline concentration, or even to the sum of both effects. In this regard, Yu and Damodaran (1991) have reported in a previous work that a slight increase of the ionic strength in the 0−0.1 M range leads to a significant increase in the foam stability. In the present work, the NaCl content of the aqueous solutions under study lies within that range (in the sample treated at pH 1, the solution having 10 mg of isolate/mL had a maximum NaCl concentration of 0.045 M). In order to eliminate the effect of a variable NaCl concentration, we studied the foaming properties of isolates in solutions of 0.5 M NaCl. In all isolates, the ionic strength was $\mu = 0.500 \pm 0.045$. Thus, we can analyze the influence of μ on proteins of different denaturation degree at constant saline concentration. The effect of a high μ on v_i is greater in the native isolate, but, even so, we still observe differences between such an isolate and the modified one, the differences being more evident at high concentrations (compare parts a and c of Figure 9). With regard to stability, isolate concentration levels ($\geq$2 mg/mL), that in aqueous medium lead to differences between native and modified isolates, produce, in a medium of high ionic strength, foams of minimum K values (compare parts b and d of Figure 9). For isolate concentrations $\leq$1 mg/mL, only the isolate modified at pH 1.0 reached the maximun stability value, even though the saline effect was eliminated. Yu and Damodaran (1991) have indicated that at such a saline level the effect of ionic strength variation is almost null. Then, differences between pH 1 treated isolate and other ones would be attributed only to protein modification

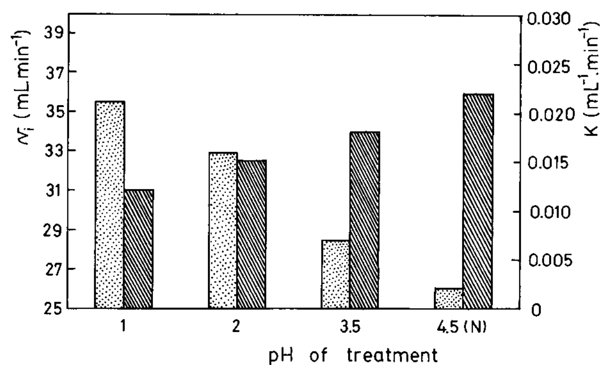

Figure 10. Rate of liquid incorporation into the foam (v_i, stippled bars) and specific rate constant of drainage (K, hatched bars) as a function of pH of treatment. Foaming assays were performed in 0.5 M NaCl dispersions of native (N) and treated isolates at 0.5 of mg protein/mL.

by acid medium. High ionic strength conditions (Figure 9c,d) improve the foam formation and lead to v_i and K values similar to those of aqueous dispersions at concentrations 10 times smaller of the same isolate (Figure 9a,b).

Under the conditions in which the foaming tests were carried out, the maximum foam density ($D_{max} = 111.3$) was reached once all the liquid was incorporated into the foam. The treatments in acid medium modify the concentration required to reach such a value (maximum density), which is a measure of foam quality. Therefore, in aqueous solutions, all modified isolates require concentrations of 5 mg/mL to reach such D_{max} whereas in native isolates that value is not reached even at a concentration of 10 mg/mL. In 0.5 M NaCl solution, isolates modified at pH 1−2 reached the D_{max} value at concentrations of 1−2 mg/mL; at pH 3.5, a concentration of 5 mg/mL is required, whereas with the native isolate the D_{max} value is reached at concentrations not below 10 mg/mL. This would be caused by the high ionic strength which, in turn, favors hydrophobic interactions with a stabilizing effect on the protein lamella (Kim, 1985), this effect being stronger on the modified isolates.

In order to eliminate variations due to different protein concentrations between modified and native isolates, isoconcentration foaming tests (at 0.5 mg of protein/mL) at high and constant ionic strength were performed. In these conditions, the effect of the acid treatment led to more marked differences in foam formation and stability (Figure 10). Thus, it is demonstrated that the variation of the surface properties is really due to modifications in the denaturation−dissociation state of proteins in the isolate, mainly of globulin 11S, the modifications, in turn, being induced by acid medium action. This would explain the low density and stability of the foams obtained in the untreated isolate, in which protein 11S is in its native oligomeric state of high molecular size (molecular mass = 360 kDa; Peng et al., 1984). Once denatured and dissociated at acid pH, this protein would be more easily adsorbed in the water−air interphase (Kim, 1985), in which the protein would tend to form aggregates caused by hydrophobic interactions promoted, in turn, by its high surface hydrophobicity (Figure 6). Townsend and Nakai (1983) have found a significant correlation between the foaming capacity and the hydrophobicity (measured by fluorometry), only when proteins in the solution were totally unfolded. Horiuchi et al. (1978) have related foam stability to surface hydrophobicity or hydrophobic regions in a protein molecule. In the

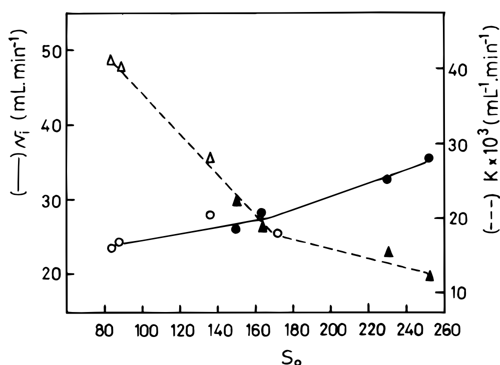

Figure 11. Relationship between v_i (○, ●) and K (△, ▲) values and ANS surface hydrophobicity (S_0) of aqueous dispersions of dialyzed isolates (○, △) and 0.5 M NaCl dispersions of nondialyzed isolates (●, ▲).

present work, we found correlations between S_0 and the formation (v_i) and stability (K) of the foams obtained with the modified isolates (Figure 11). Such curves are valid for isolate solutions (dialyzed or not) of low (μ < 0.05) or high ionic strength (μ > 0.5) since we included values obtained from tests performed in both water and solutions of 0.5 M NaCl. Although there are some other parameters that influence foam formation and stability, e.g., molecular size and flexibility (which also undergo modifications during the acid treatment), it was observed that the surface hydrophobicity plays an important role in the foaming properties. The influence of the latter is much stronger on the stability, since a 2-fold increase in S_0 (from 80 to 160) makes the K value decrease 2.1 times. Higher S_0 values do not improve significantly the foam stability, which is reflected by the change of the slope. On the contrary, v_i increases only 1.5 times in the whole S_0 range.

Unlike thermal and enzymatic treatments (Kinsella et al., 1985), the modifications induced by the acid effect do not influence the gelation capacity of the isolates. It was observed that the aqueous solutions of all modified isolates form gels by heating at 100 °C for 15 min at the same concentration (9% w/w) as that of the native isolate. At present, the effect of modifications induced by acid treatment on the rheologic properties of the gels formed is under study. Recent studies in our laboratory show that heat-induced gels can be obtained in acidic conditions (Puppo et al., 1995).

As a conclusion, the acid treatment at several pHs allowed us to obtain modified isolates of considerably different structures. By varying treatment conditions, high-solubility isolates can be obtained, but to achieve this, the treatment should be performed on a native isolate obtained from a defatted soybean flour without prolonged previous storage. Otherwise, the treatment should be done at a pH no lower than 3.5, at low protein concentrations (≤2%), and with rapid neutralization. None of the treatment conditions would permit us to improve significantly the water imbibing capacity of the isolate.

The results obtained demonstrate that treatment in acid medium that leads to modified isolates of high solubility improved the foaming properties without losing the gel formation capacity. Such characteristics confer the isolates more versatility for their use in food formulation. This isolates are expected to be good emulsifying agents owing to improvements in their surface properties as a consequence of acid treatment. The results of a current in-depth study about the emulsifying and gelling properties of modified isolates are to be presented in a future publication.

CONCLUSIONS

The high sensitivity of glycinin (soy protein 11S) to the denaturing action of the acid medium possibilitates its selective denaturation, the degree of which is regulated by pH and time of treatment.

The acid treatment leads to loss of solubility only in those isolates extracted from flours that were previously stored for long periods. The presence of the insoluble fraction can be attributed to insolubilization of previously-existing aggregates (forming during storage of the flours) by effects of pH and ionic strength of the medium. There are equal distributions of denatured proteins in soluble and insoluble fractions, which suggest nonselective protein aggregation during acid treatment.

Even though structural changes were evident, the improvement of functional properties was limited. The variables of the acid treatment (pH, time, neutralization procedure, and isolate concentration) modify lightly isolate solubility but do not alter the water imbibing capacity (WIC) in a significant way, even when the insoluble fraction possesses a high WIC.

Isolates modified in acid medium have greater capacity to form and to stabilize foams than the native isolates. This is mainly caused by the high degree of denaturation and dissociation of 11S protein (which lead to reduced molecular size and to increased surface hydrophobicity) (Wagner and Guéguen, 1995) and by the high saline content due to previous neutralization.

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