

# Rheological Study of Dispersions Prepared with Modified Soybean Protein Isolates

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**ABSTRACT:** Structural modifications of modified soy protein isolates (SPI) were distinguished by rheological behavior. SPI were prepared by acidic (pH 2.5) and thermal-acidic treatment without (pH 1.6) and with neutralization (pH 8.0). Dynamic properties of dispersions were determined through the variation of storage and loss moduli with frequency, and loss tangent behavior was analyzed. Changes in viscoelastic parameters with protein concentration (10–12% wt/vol) and time of heating (15–60 min) were also determined. Flow properties of dispersions were estimated through apparent viscosity and flow and consistency index measurements. Rheological behavior of dispersions was compared with those found by experiment with commercial mayonnaise, mustard, and salad dressing. The analysis of rheological parameters showed that thermally treated isolates formed dispersions with high elastic modulus and consistency index with a structure mainly stabilized by hydrophobic interactions, although no gelation process after cooling was observed. From the rheological point of view, it was deduced that thermally treated isolates could be used as ingredients in the formulation of salad dressings. The alkaline sample would be more versatile because, depending on protein concentration and thermal treatment, the consistency of its dispersions was like that in salad dressing, or similar to those of mustard and mayonnaise.

Paper no. 9318 in *JAOCs* 77, 63–71 (2000).

**KEY WORDS:** Apparent viscosity, modified soybean isolates, salad dressing, viscoelasticity.

Soybean protein isolates (SPI) have been included in a wide variety of formulated foods because of their high nutritional value and desirable functionality (1). Functional properties of isolates reflect the composition, structure, denaturation, and degree of aggregation of their major components: 11S (glycinin) and 7S ( $\beta$ -conglycinin) globulins (2,3). The different treatments applied during SPI extraction and/or processing cause physical and chemical changes in the protein (4). Appropriate modifications produce suitable functional properties and improve the isolates' use as ingredients in the food industry. Thermal treatments induce dissociation, denaturation, and aggregation of 7S and 11S (5–7), whereas acid treat-

ments lead to denaturation and selective dissociation and unfolding of 11S with lesser effect on 7S and minimal protein aggregation (8–10). Thermal-acidic treatments introduce additional modifications such as hydrolysis and deamidation (11,12).

Modifications in the denaturation degree and aggregation state of proteins are reflected both in solubility and in water-imbibing capacity (WIC). The insoluble fraction is responsible for most of the WIC of isolates, which is directly related to viscosity, whereas other properties such as gel- and foam-forming capacity depend mainly on the soluble fraction (13). A possible use of these modified isolates is as ingredients of low-calorie salad dressings. Generally, salad dressings are acidic oil/water emulsions containing vinegar, salt, and egg as emulsifiers. They differ from mayonnaise in that they also contain starch paste as thickener (14). Oil (in mayonnaise) and starch (in salad dressing) are the major sources of viscosity and body. Egg yolk is the major emulsifier and contributes by lowering the oil/water interfacial tension (15). Egg white protein, which cannot be totally separated from yolk without difficulty, favors emulsification by forming a solid gel structure on being coagulated by the acid component (16).

The rheological behavior of a protein system is directly related to the structural and functional characteristics of the different components. The structural and functional properties of native soy isolates and of different isolates modified by thermal and acid treatments were analyzed in previous work conducted in our laboratory (7,10,13,17).

The aim of the present work was to analyze the structural behavior of SPI, as modified by different thermal and/or acid treatments. To this end, rheological properties of dispersions under several thermal treatment conditions and protein concentrations were studied. Various commercial samples were also characterized from the rheological standpoint, so as to evaluate the ability of these modified isolates to act as basic ingredients in salad dressings.

## MATERIALS AND METHODS

*Preparation of modified SPI.* SPI were prepared from defatted flour produced by Santista Alimentos S.A. (Porto Alegre, Brazil). An aqueous alkaline (pH 8) solubilization was conducted for 2 h at 20°C, followed by centrifugation at 6,000  $\times$

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g for 30 min at 10°C. Supernatant proteins were precipitated at the isoelectric point (pH 4.5) and centrifuged at 5,000 × g for 15 min at 20°C. The native isolate (N) was obtained by preparing a water suspension of the pellet and by bringing the pH to 7. To prepare the different modified isolates, the isoelectric precipitate was resuspended in water to a protein concentration of 5% w/w, under acidic pH and thermal treatment conditions: (i) Acid treatment: the isoelectric precipitate dispersion was brought to pH 2.5 to obtain the isolate H2.5. (ii) Acid thermal treatment: the dispersion was adjusted to pH 1.6 and heated to 90°C for 30 min to obtain the isolate TH1.6; a fraction of the thermally treated pH 1.6 dispersion was neutralized to pH 7, to obtain the isolate TH1.6N. The pH adjustments were done using 0.5 M HCl and NaOH as needed. Dispersions were frozen and freeze-dried.

The different denaturation and/or aggregation degree and hydration properties were described in a previous work (18) and are summarized in Table 1.

**Preparation of soy protein dispersions.** Aqueous dispersions at 10 and 12% w/w protein concentration of modified isolates H2.5, TH1.6 and TH1.6N were prepared. An aliquot from the dispersions was taken and exposed to thermal treatment followed by cooling. To this end, samples were placed in glass tubes (2.2 cm i.d. × 6 cm height) tightly closed with stoppers and then were heated at 90°C for 15, 30, or 60 min and cooled for 10 min in a water bath at 15°C and then 48 h at 4°C.

**Commercial samples.** (i) Salad dressing (SD) (Hellman's, Refinerías de Maíz S.A., Argentina) contains vegetable oil, water, vinegar, sugar, spices, yeast extract, microcrystalline cellulose and xanthan gum as stabilizers, β-carotene as coloring, natural flavor enhancers, and emulsifiers. (ii) Mayonnaise (MY) (Ri-k, Molinos Rio de La Plata S.A., Argentina) consists of sunflower oil, pasteurized liquid egg, water, vinegar, sugar, salt, lemon juice, citric acid, spices, preservatives, antioxidants, and β-carotene as coloring. (iii) Mustard (MS) (Dánica, Flora San Luis S.A., Argentina) is composed of water, vinegar, sugar, black and white mustard seeds, salt, starch, and spices.

**Viscoelasticity.** Tests were carried out in a Haake CV20 rheometer (Karlsruhe, Germany) using a 1-mm gap parallel-plate sensor. The dispersion was placed on the lower plate, which was maintained at 20 or 90°C. For measurements at 90°C, low-viscosity silicone was added around the plate

edges to prevent sample dehydration. The equipment was driven through the Haake software osc. 2.0. Experimental data were obtained by recording the complex modulus ( $G^*$ ) as a function of deformation and the storage modulus ( $G'$ ), loss modulus ( $G''$ ), and  $\tan \delta$  as functions of oscillation frequency ( $f$ ).  $\tan \delta$  is represented by Equation 1,

$$\tan \delta = \frac{G'}{G''} \quad [1]$$

The linear viscoelasticity range of the dispersions was determined by measuring  $G^*$  as a function of deformation ( $f = 1$  Hz). From these results, frequency scans of the samples were conducted at the same deformation ( $d = 8\%$ ), within the linear range. The dynamic behavior ( $G'$  and  $G''$  vs. frequency) of the dispersions was studied in different thermal treatment conditions: without heating, during heating, and with thermal treatment followed by cooling. Measurements were done at 20°C in unheated samples and in those exposed to thermal treatment and cooling. Concerning the heating stage, samples were first heated *in situ* at 90°C for 30 min to measure rheological parameters at this temperature.

**Apparent viscosity ( $\eta_{app}$ ).** The  $\eta_{app}$  was measured in unheated samples and in those thermally treated and then cooled. The torque ( $S$ ) was measured at 20°C in a Haake Rotavisco RV2 viscosimeter with an NV sensor system. This system consists of a coaxial cylinder with two gaps (inner gap = 0.35 mm, outer gap = 0.4 mm). The rotation rate was increased from 0 to 128 rpm in 2 min, and maintained 1 min at maximum speed. The  $\eta_{app}$  was calculated taking into consideration the instrument factors  $G$  (cP/degrees of the scale × min), the degree of the scale  $S$  and the rotor velocity  $n$  (rpm) (Eq. 2):

$$\eta_{app} = \frac{GS}{n} \quad [2]$$

**Statistical analysis.** Each measurement was carried out in duplicate. Data were analyzed by analysis of variance (ANOVA) using the SYSTAT software (1990) (SYSTAT, Inc., Evanston, IL). The significance of differences among the results of several treatments was studied by the Tukey test at  $P < 0.05$ . Difference between means ( $\Delta_{0.05}$ ) for each rheological parameter was calculated.

**TABLE 1**  
Denaturation Enthalpy ( $\Delta H$ ), Water-Imbibing Capacity (WIC), Solubility (S), Surface Hydrophobicity ( $H_0$ ), High Molecular Weight Aggregates (HMW), and Hydrolysis of Native and Modified Soy Protein Isolates (from Ref. 18)

Isolate <sup>a</sup>	$\Delta H$ (J/g)	WIC (mL/g)	S (%)	$H_0$	HMW	Partial hydrolysis
H2.5	1.84	6.34	77.5	235.8	+ <sup>c</sup>	++ <sup>c</sup>
TH1.6	0	7.63	65.0	> 300	+ <sup>c</sup>	++ <sup>d</sup>
TH1.6N	0	17.40	38.5	355.6	++ <sup>d</sup>	+ <sup>c</sup>
N	16.37	7.06	75	164	— <sup>b</sup>	— <sup>b</sup>

<sup>a</sup>See Materials and Methods section for description of codes describing preparation of modified soy protein isolates.

<sup>b</sup>Not present.

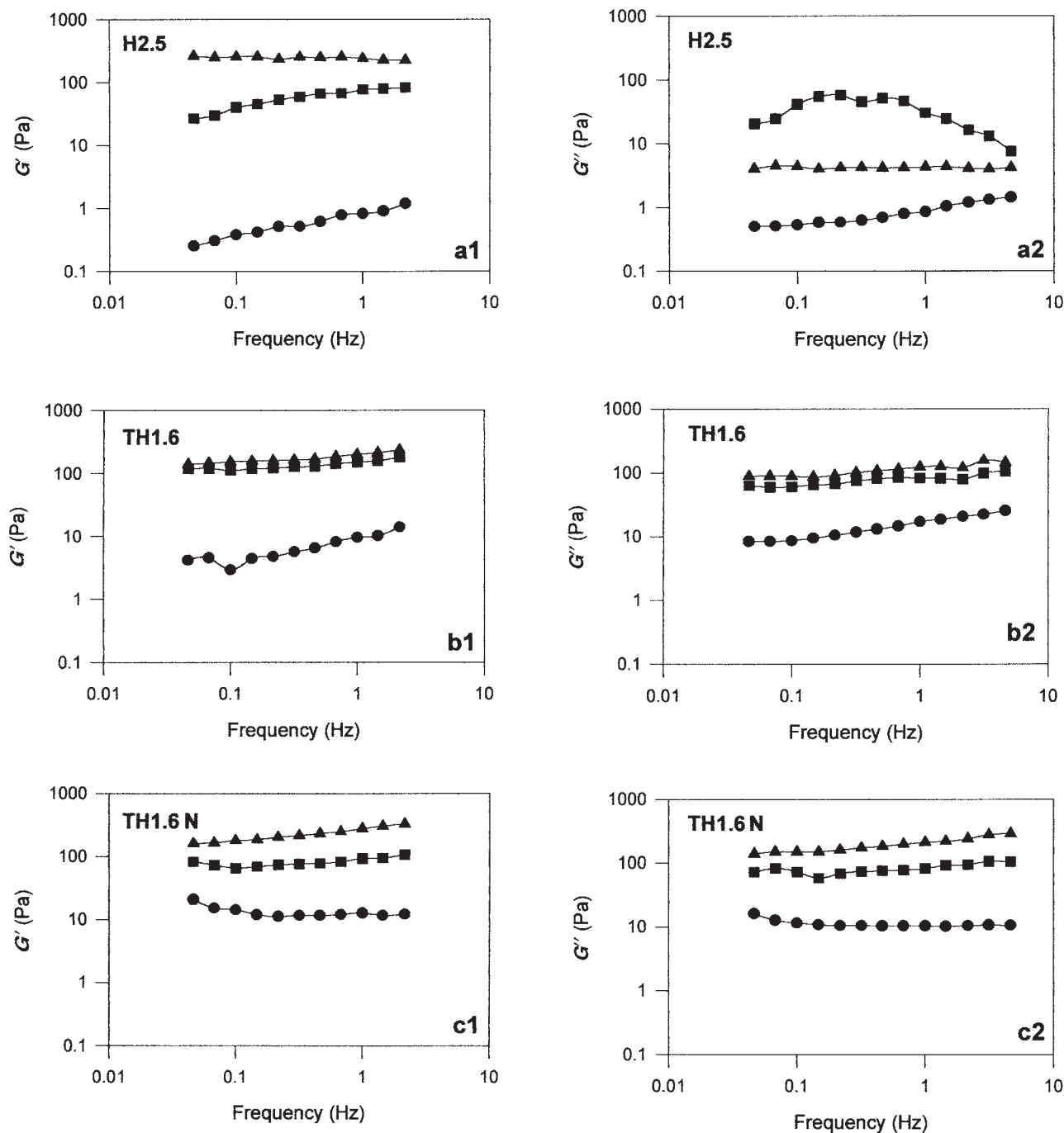
<sup>c</sup>Present

<sup>d</sup>Present in higher proportion

## RESULTS AND DISCUSSION

Viscoelasticity of isolates modified by acid treatment (H2.5), or thermal-acid treatment (TH1.6 and TH1.6N), is studied here by analyzing the variations of the elastic ( $G'$ ) and viscous ( $G''$ ) moduli as a function of the oscillation frequency. The effect of the different thermal treatments on the  $G'$  and

$G''$  values was analyzed for the different isolate dispersions at 10% w/w (Fig. 1). For the H2.5 isolate, we observed, at 20°C, a liquid protein dispersion where  $G'$  slightly increased with frequency, showing values below 1 Pa (Fig. 1 a1). Values and behavior of the viscous modulus were similar (Fig. 1 a2). During heating stage at 90°C,  $G'$  increased from  $\approx 20$  to 80 Pa at  $\approx 0.7$  Hz frequency, remaining constant at higher fre-



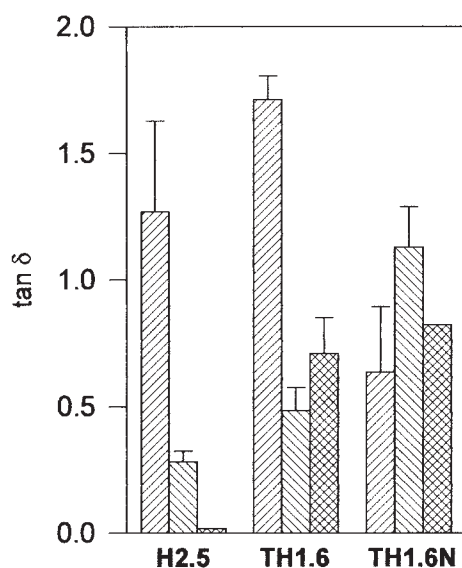
**FIG. 1.** Dynamic oscillatory rheological analysis. Frequency dependence of storage modulus  $G'$  (a1, b1, c1) and loss modulus  $G''$  (a2, b2, c2) of 10% w/w dispersions of H2.5 (a), TH1.6 (b), and TH1.6N (c) isolates. Sample treatments: (●) unheated, (■) heating *in situ* (90°C—30 min), (▲) heated (90°C—30 min), and cooled (4°C—24 h); measurements done at 20°C (●, ▲) and 90°C (■). See Materials and Methods section for codes describing preparation of modified soy protein isolates.

frequencies. Under 0.3 Hz,  $G''$  was almost equal to  $G'$ , decreasing ( $G'' < G'$ ) at increasing frequencies. After cooling, it was observed that although  $G'$  and  $G''$  stayed constant for increasing frequencies, the  $G'$  values increased up to  $\approx 300$  Pa, whereas  $G''$  decreased to  $\approx 5$  Pa (Fig. 1 a1 and 1 a2, respectively). It is well known that heating mainly stabilizes hydrophobic bonds, and hydrogen bonds are stabilized with decreasing temperature (19). The marked elasticity increase induced by cooling the sample, evidenced by the increase in  $G'$  and simultaneous noticeable decrease in  $G''$ , would be caused for the formation of a translucent gel, mainly stabilized by hydrogen bonds. The unheated TH1.6 sample (Fig. 1 b1), as well as the H2.5, exhibited a slight increase of  $G'$  with frequency, but reaching higher values ( $>8$  Pa). In the whole frequency range tested,  $G'$  remained below  $G''$  (Fig. 1 b2), indicating viscous-liquid behavior. During heating and after cooling, the structures formed by TH1.6 dispersions were rheologically similar, with high  $G'$  values ( $\approx 100$  Pa) and slightly lower values of  $G''$ , both independent of frequency. These results indicate that the structure is basically stabilized by hydrophobic bonds, so, in this case, no hydrogen bond formation was detected after cooling. In TH1.6N samples (20°C), of liquid consistency, the  $G'$  did not vary with frequencies above 0.1 Hz, and its values ( $\approx 10$  Pa) were higher than in H2.5 and TH1.6 acid samples (Fig. 1 c1). Heating induced a considerable increase in  $G'$  ( $\approx 100$  Pa), which intensified during cooling, reaching values  $>200$  Pa. A granulose, opaque dispersion was formed, which was firm to the touch, especially after cooling. Regardless of the thermal treatment, the viscous component ( $G''$ ) stayed constant with frequency in values similar to that of the elastic modulus, suggesting an important viscous contribution (Fig.

1 c2). In turn, variation of  $G'$  indicates that hydrophobic interactions and other noncovalent bonds (mainly hydrogen bonds) are important, though, in this case, the possible contribution of disulfide bonds favored by the neutral pH of the sample should not be discounted.

Viscoelastic behavior can be better evidenced by  $\tan \delta$ , a rheological parameter that relates the viscous with the elastic behavior (Eq. 1). Figure 2 shows  $\tan \delta$  values (1 Hz, 8% deformation) of the different samples and treatments. The H2.5 dispersion values (1.27) decreased significantly on heating, and even to a greater extent after cooling for 48 h at 4°C, to reach values  $<0.1$  ( $P < 0.05$ ). These results would be influenced by the small fraction of proteins that retain their native state at pH 2.5 (10), so the dispersion is able to form gels on heating even in the absence of subsequent cooling. This behavior confirms the transformation of the H2.5 dispersion from the sol to the gel state. Previous studies showed that soy proteins denatured only by acid treatment exhibited high elasticity during heating (90°C, 30 min) (20). Thermal treatment favors the formation of a structure consisting mainly of hydrophobic bonds that stabilize during cooling by formation of hydrogen bonds. The rheological behavior is directly related to the isolates' structural properties described in Table 1. These proteins are composed of the totally denatured and dissociated 11S fraction and of the partially denatured 7S fraction (10). The proteins are positively charged at acidic pH, thus favoring intermolecular repulsion and protein-water interactions. The isolate so obtained possesses high solubility, with similar WIC and greater surface hydrophobicity ( $H_0$ ) compared to native isolate (Table 1), and can form a matrix by thermal treatment. Previously, studies were conducted on the solubility of acid soy protein gels in different extraction media, and of the corresponding soluble fractions. Gels were found to have a matrix formed both by 7S and 11S fractions, but mainly stabilized by noncovalent bonds (hydrophobic and hydrogen bonds) of the 7S globulin (17). Both the gradual increase of  $G'$ , first in the heating stage (hydrophobic bond stabilization), and then during cooling (hydrogen bond stabilization), and the decrease in  $\tan \delta$  (Fig. 1A and 2) confirm the nature of gel matrix.

In the absence of heating, the TH1.6 dispersion showed a high  $\tan \delta$  (1.71), which decreased considerably during heating (0.50) (Fig. 2). No significant changes ( $P < 0.05$ ) in  $\tan \delta$  were observed after the dispersion was cooled, suggesting the formation of a structure that remains stabilized only by the hydrophobic interactions developed during heating. This fact is directly related to the structural modification in the isolate. Heating at 90°C for 30 min denatures the TH1.6 isolate, so conferring to it a high  $H_0$ , and lower solubility and greater WIC compared to N and H2.5 isolates (Table 1). Matsudomi *et al.* (11) observed that no hydrolysis occurred, and deamidation degree did not surpass 10% when an acid protein dispersion (pH 1.6) was heated at 90°C for 30 min. In these conditions, the protein had maximum  $H_0$ , and its isoelectric point did not differ from the native isolate value ( $pI = 4.5$ ). The positive charge of TH1.6 isolate along with its elevated  $H_0$  pro-



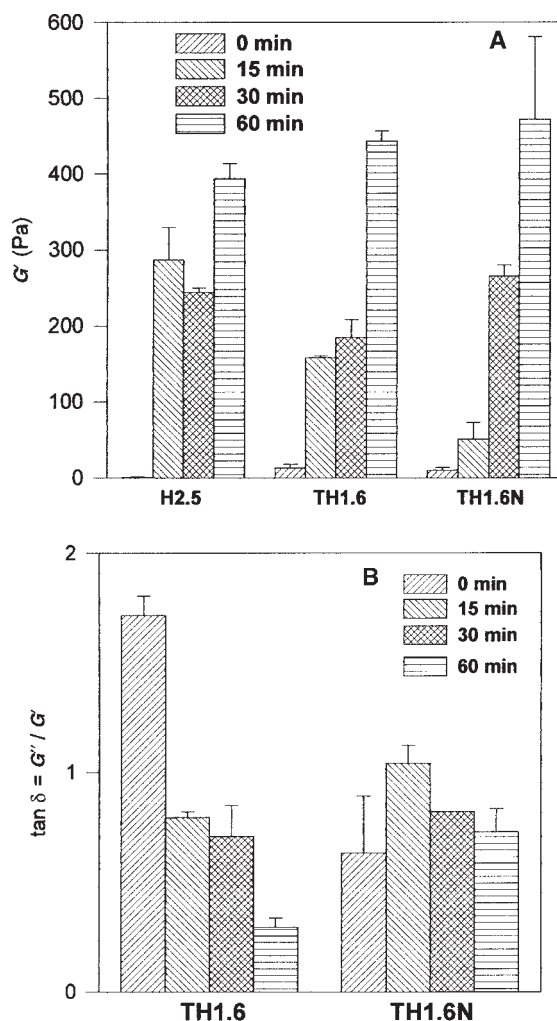
**FIG. 2.** Loss tangent,  $\tan \delta$  ( $G''/G'$ ) of modified soybean protein isolates (10% w/w dispersions). Samples: (lines trending up) unheated, (lines trending down) heating *in situ* (90°C—30 min), (cross-hatched) heated (90°C—30 min) and cooled (4°C—24 h). Calculated difference between means at  $P < 0.05$ ,  $\Delta_{0.05} = 0.686$ . See Materials and Methods section for sample codes.

motes the formation of a matrix stabilized only by hydrophobic bonds.

At room temperature,  $\tan \delta$  in TH1.6N isolate was  $\approx 0.6$ , owing to the important contribution of the elastic component. This behavior is caused by negatively charged high molecular mass protein aggregates (Table 1), which tend to form a structured dispersion as a consequence of salt addition during neutralization. The presence of these aggregates markedly decreases the isolate solubility, while the increase of the insoluble fraction shifts the WIC to higher values. At variance with the results observed in H2.5 and TH1.6 isolates, no significant changes in  $\tan \delta$  were observed on heating and subsequent cooling, suggesting that viscous and elastic components were similarly modified by the thermal treatment.

An attempt was made to determine whether the structural changes detected in the dispersion *via* rheological behavior were sensitive to the time of heating. Therefore, for the different dispersions, the effect of heating time (0–60 min) on the variation of  $G'$  and  $\tan \delta$  was analyzed. A marked increase of  $G'$  was observed in acid dispersions after only 15 min of heating by turning the sample from liquid ( $G' \approx 0$ ) to a gel state (Fig. 3A). Longer heating times did not modify  $G'$  any further in H2.5 dispersions. In turn, an increase of  $G'$  with time was observed in TH1.6 and more evident in TH1.6N samples ( $P < 0.05$ ). The elastic contribution to the structure formed by all dispersions was high after 60 min of heating. The H2.5 dispersions passed from the sol ( $\tan \delta \approx 1$ ) to the gel state ( $\tan \delta \approx 0.1$ ) with only 15 min of heating, and no further differences among values of  $\tan \delta$  were observed for longer heating times (data not shown). The unheated TH1.6 dispersion showed a significantly high  $\tan \delta$  value (1.71), suggesting that  $G''$  predominates over  $G'$  ( $P < 0.05$ ). The nature of these dispersions changed on heating, turning from semiliquid to more creamy consistency.  $\tan \delta$  decreased substantially with 15 min of heating, and especially after 60 min. This  $\delta$  variation in  $\tan$  with heating time is directly related to the marked increase in  $G'$ , since  $G''$  did not change to any important extent (Fig. 3A and B).  $\tan \delta$  values were not significantly different ( $P < 0.05$ ) among TH1.6N dispersions unheated or heated for different times because the marked increase in  $G'$  was paralleled by that of  $G''$  (Fig. 3A and B).

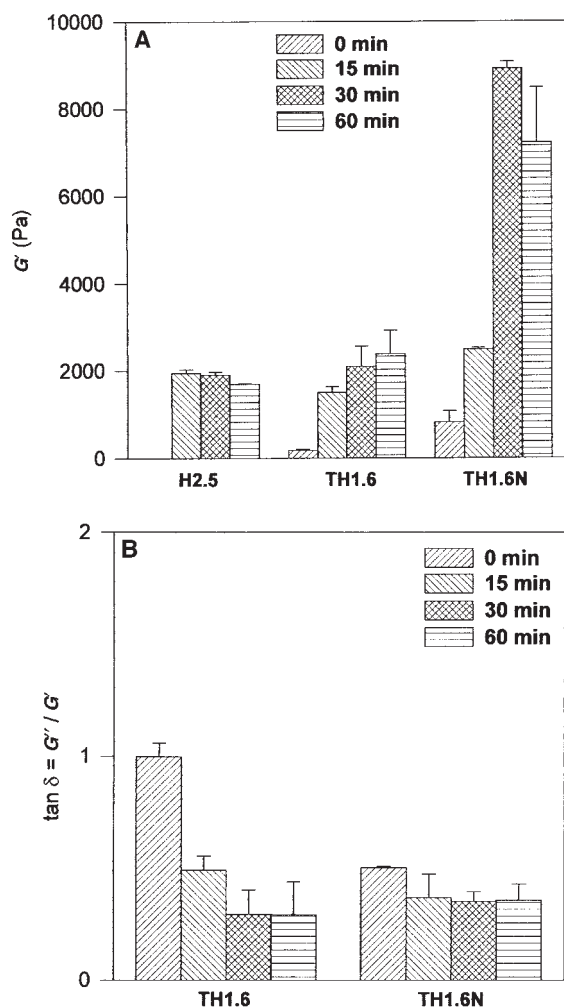
An increase in protein concentration from 10 to 12% w/w caused a great increase of  $G'$  values in all samples, especially in TH1.6N (Fig. 4A). In the H2.5 and TH1.6 dispersions, the stronger protein–protein interaction permitted, with only 15 min of heating, very high  $G'$  values that were independent of heating time and higher than those observed at 10% wt/wt protein concentration. TH1.6N was the only sample that presented a significantly different ( $P < 0.05$ ) rheological behavior for increasing protein concentration.  $G'$  increased sharply during the first 30 min, reaching very high values (8915 Pa) (Fig. 4 A). Regardless of heating time, the matrix of gels formed by 12% dispersions of the H2.5 isolate was much more elastic than in 10% gels ( $\tan \delta < 0.05$ ) (data not shown). This phenomenon, called thermotropic gelation, is a process whereby gels are formed by heating concentrated protein so-



**FIG. 3.** Effect of heating (90°C) time on  $G'$  (storage modulus) (A) and  $\tan \delta$  (B) of 10% w/w soybean protein isolates (SPI) dispersions. (A)  $\Delta_{0.05} = 143$ , (B)  $\Delta_{0.05} = 0.501$ . See Figure 2 caption for  $\Delta$ , and Materials and Methods section for sample codes.

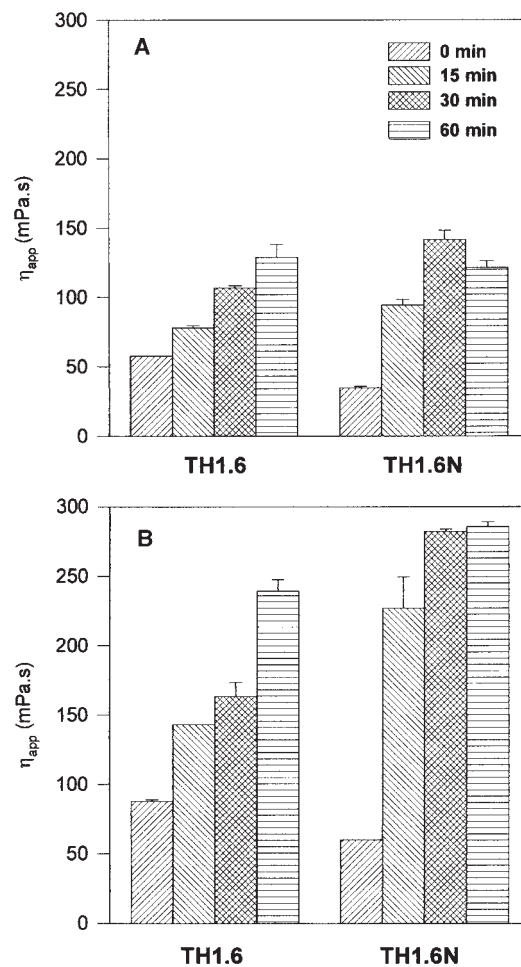
lutions. Grinberg *et al.* (21) have suggested that denatured molecules tend to reestablish their initial structure and rearrange to form the gel matrix. From previous studies, it is known that at pH 2.5 and 10% w/w protein, heating produces total and partial denaturation of the 11S and 7S soy protein fraction, respectively, favoring the gelation process after cooling (10). The same behavior as at 10% with increasing heating time was observed for  $\tan \delta$  of TH1.6 and TH1.6N dispersions at 12% w/w but with lower values ( $\approx 0.4$ ) (Fig. 4B). As protein concentration increases, the thermal treatment allows more elastic structures to be formed.

The  $\eta_{app}$  is a measure of the resistance to flow when a shear stress is applied to the sample at given shear rate, and would be related to the sample viscoelasticity. The  $\eta_{app}$  was measured in unheated dispersions of TH1.6 and TH1.6N isolates prepared at 10 and 12% w/w as well as in thermally treated and cooled samples (Fig. 5). In TH1.6 and TH1.6N (10% w/w) dispersions, the  $\eta_{app}$  gradually increased with thermal treatment and heating time (Fig. 5A). The  $\eta_{app}$  val-



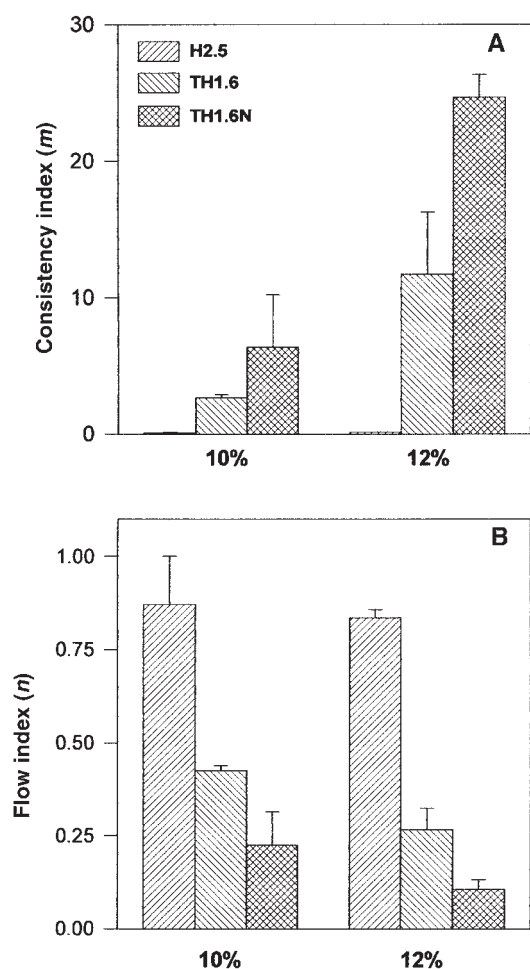
**FIG. 4.** Effect of heating (90°C) time on  $G'$  (A) and  $\tan \delta$  (B) of 12% w/w SPI dispersions. (A)  $\Delta_{0.05} = 1763$ , (B)  $\Delta_{0.05} = 0.331$ . See Figures 2 and 3 for abbreviations, and Materials and Methods section for sample codes.

ues changed more with heating time in the TH1.6N sample (Fig. 5A). In the absence of heating, increased concentrations lead to higher viscosity in the dispersions (Fig. 5B). Strengthened protein–protein interactions combined with the thermal treatment applied during isolate preparation would produce protein aggregates of high hydrodynamic volume, which are responsible for the high viscosity (22). Along with the increase in concentration, the  $\eta_{app}$  of dispersions increased significantly with thermal treatment (Fig. 5B). For the TH1.6 dispersion,  $\eta_{app}$  experienced a strong increase with 60 min of heating, whereas, in the TH1.6N dispersion, a comparable increase was observed after 15 min (Fig. 5B). The unheated TH1.6 and TH1.6N dispersions presented a pseudoplastic flow behavior with thixotropic effect together with greater hysteresis loops at higher protein concentration. Rupture or deformation of hydrated aggregates can cause the thixotropic effect, because protein concentration (made up by totally denatured proteins in these isolates) was high enough to permit association into stable aggregates, which do not break easily



**FIG. 5.** Effect of heating (90°C) time on apparent viscosity,  $\eta_{app}$  of 10% w/w (A) and 12% w/w (B) SPI dispersions. (A)  $\Delta_{0.05} = 23.2$ , (B)  $\Delta_{0.05} = 36.5$ . See Figures 2 and 3 for abbreviations, and Materials and Methods section for sample codes.

under shear stress. Two regions can be distinguished in the rheograms: rheodestruction behavior below a shear rate of  $303 \text{ s}^{-1}$  and above that value where rheologic behavior approximated that of a pseudoplastic fluid that follows the Ostwald–De Waele Model or power law. This law was applied to the rheograms of nonheated protein dispersions to calculate the indices of flow behavior ( $n$ ) and consistency ( $m$ ). Figure 6 shows that the unheated dispersions of TH1.6 and TH1.6N isolates were the samples that presented the highest consistency index ( $m$ ). This index increased in these dispersions for increasing protein concentration, at variance with the H2.5 dispersion, whose consistency did not change. Compared with  $m$ , the flow index ( $n$ ) showed an opposite behavior: in the H2.5 dispersions, its value was  $\approx 0.8$  at both protein concentrations, like that of a Newtonian fluid ( $n = 1$ ) (Fig. 6). The  $n$  values presented by TH1.6 and TH1.6N dispersions were significantly ( $P < 0.05$ ) low ( $< 0.4$ ), especially at 12% w/w protein. The TH1.6N sample showed the lowest flow index. If the  $G'$  values of unheated samples are observed (Fig. 3A and 4A), a direct correlation becomes evident between the



**FIG. 6.** Effect of protein concentration on consistency ( $m$ ) and flow ( $n$ ) indices of unheated SPI. Samples: (lines trending up) H2.5, (lines trending down) TH1.6, (cross-hatched) TH1.6N. (A)  $\Delta_{0.05} = 10.1$ , (B)  $\Delta_{0.05} = 0.281$ . See Figures 2 and 3 for abbreviations. See Materials and Methods section for sample codes.

consistency index and the elastic modulus. The H2.5 dispersions, for which the  $m$  value was very low, were in a liquid state because this isolate possesses a protein fraction that preserves its native state with high solubility and WIC like that of the native isolate (Table 1). The unheated TH1.6 and TH1.6N, with higher  $m$  and  $G'$  compared to those observed in the H2.5 sample, were able to form dispersions showing some initial structure (creamy), possibly because of the high molecular mass aggregates they contained. The thermally treated H2.5 dispersions formed a gel with a stable matrix, whereas the structure presented by TH1.6 and TH1.6N revealed a nonpseudoplastic behavior, for which the power law cannot be applied to determine the  $m$  and  $n$  indices. Notwithstanding, the consistency of these samples could be evaluated *via* the  $G'$  modulus.

A possible application of soy isolates is as ingredients of dressings. Three acid dressings of different consistency were selected as references to analyze the rheological parameters of modified soy protein dispersions. The dressings were SD (liquid consistency), MY (creamy), and MS (intermediate).

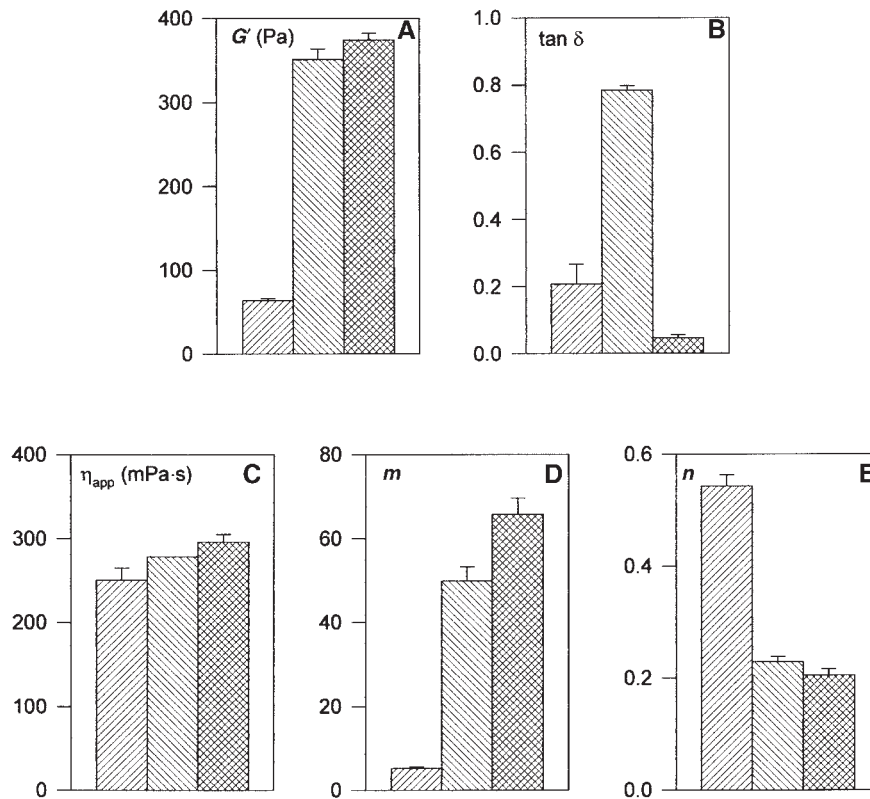
Both MY and SD have been widely studied by several authors (14,23,24). Rheological parameters  $G'$ ,  $\tan \delta$ ,  $\eta_{app}$ ,  $m$ , and  $n$  of the commercial samples were analyzed and results are shown in Figure 7. The elastic modulus  $G'$  (8% deformation, 1 Hz frequency) was considerably lower in SD than it was in MY and MS.  $G'$  in the last two dressings were not significantly different ( $P < 0.05$ ) (Fig. 7A). Mayonnaise presented the lowest  $\tan \delta$  (0.1), so its viscoelastic behavior was similar to that of gels; on the contrary, viscous behavior predominates in mustard (Fig. 7B). The  $\eta_{app}$  was similar in the three dressings (Fig. 7C). All samples presented pseudoplastic flow behavior, the thixotropic effect being present in MY and MS. The power law was used to calculate the consistency and flow indices (Fig. 7D and E). Unlike MS and MY, the SD had low consistency and high fluidity; MY had the highest consistency and, like MS, had very low flow index values.

In each food, the rheological behavior is directly related to its formulation: the conformational changes experienced by egg lipoproteins in MY and by starch in MS would be largely responsible for the high resistance to flow, high consistency, and predominantly elastic behavior. In MY, the underlying phenomena that determine the observed rheological behavior would be denaturation, unfolding, and interaction of lipoproteins with other components such as lipids during pasteurization (14). In MS, the main process would be starch gelatinization. The absence of macromolecules such as proteins and starch would determine the viscous behavior of SD.

The  $G'$  values of unheated modified soy isolate dispersions prepared at 10% w/w were low compared to those presented by the commercial samples. The increase of protein concentration to 12% w/w did not modify the  $G'$  modulus of the H2.5 sample, but caused an increase of  $G'$  of the TH1.6 dispersion (172 Pa) to values closer to those in MY (374 Pa) and MS (351 Pa), whereas the  $G'$  of TH1.6N (817 Pa) largely surpassed such values. The  $G'$  values of heated, 10% dispersions were also within the range of those measured for MY and MS, but were more dependent on thermal treatment time in the TH1.6N sample. For short times (15 min),  $G'$  values of this last sample were very low, similar to those of SD, whereas, at 30 min they were within the values of MY and MS. At 12% w/w protein concentration,  $G'$  values of all heated samples were high ( $>2000$  Pa) and very superior to those of commercial products. The  $\tan \delta$  values in all 10% TH1.6N dispersions and in the 10% TH1.6 dispersions heated for 15 and 30 min were within the range of the values obtained in MS (about 0.8). For 10% TH1.6 dispersions heated for 60 min and all 12% w/w heated dispersions,  $\tan \delta$  values were like those measured for SD.

In unheated dispersions, the  $\eta_{app}$  values were lower than those of commercial samples, whereas the values shown by the thermally treated, 12% samples, particularly TH1.6N, were similar.

The most suitable conditions for soybean-based formulation with similar rheological properties to that of commercial products depend on the nature of isolate modification, protein concentration, and thermal treatment conditions.



**FIG. 7.** Rheological parameters of commercial samples: (lines trending up) salad dressing, (lines trending down) mustard, (cross-hatched) mayonnaise. (A) Storage modulus, (B) loss tangent, (C) apparent viscosity, (D) consistency index, (E) flow index. (A)  $\Delta_{0.05} = 36.8$ , (B)  $\Delta_{0.05} = 0.0846$ , (C)  $\Delta_{0.05} = 41.3$ , (D)  $\Delta_{0.05} = 12.3$ , (E)  $\Delta_{0.05} = 0.0605$ . See Figure 2 for abbreviation.

## ACKNOWLEDGMENTS

This work was supported by funds from SECYT (Secretaría de Ciencia y Tecnología) (Prog. BID 802 OC-AR/PID 0374), Argentina.

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[Received July 14, 1999; accepted September 20, 1999]