

Rheological Properties of Emulsions Containing Modified Soy Protein Isolates

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ABSTRACT: The feasibility of replacing common emulsifiers with soy protein isolates (SPI) in low-calorie salad dressings was evaluated. Structural modifications of SPI were obtained by thermal-acidic treatment with or without neutralization (TH1.6N and TH1.6, respectively). Modification of flow properties of TH1.6 and TH1.6N emulsions by thermal treatment and different protein concentrations was evaluated through shear stress vs. shear rate measurements in a rotational viscometer. TH1.6N isolates generated emulsions with higher shear stress and apparent viscosity than those prepared with TH1.6. Heated TH1.6N emulsions at 10% protein gave the highest values of shear stress and plastic flow behavior. These emulsions had high consistency, viscosity, and elasticity. TH1.6N isolates had lower emulsifying capacity than TH1.6, probably due to the higher protein aggregation produced during neutralization, which prevented protein unfolding. These isolates would be suitable for the preparation of stable emulsions with adequate consistency and elasticity.

Paper no. J10025 in *JAOCs* 80, 605–611 (June 2003).

KEY WORDS: Consistency, elasticity, modified soybean isolates, soy protein emulsions, viscosity.

The functionalities of soy protein isolates (SPI) depend on the composition, structure, degree of dissociation, denaturation, and/or aggregation of their major component (11S and 7S globulins) (1,2). Processing treatments performed on SPI may lead to specific changes in protein structure and may modify functional properties.

Structural changes may be accomplished by thermal or chemical treatments. Unfolding, denaturation, and selective dissociation of 11S with minimal effect on 7S and on protein aggregation may be obtained by acid treatment (3–5). Acidic treatment in combination with a thermal treatment may cause more denaturation and aggregation, as well as some hydrolysis and deamidation. The subsequent effects depend on the time and temperature of heating (6), protein concentration, pH, and ionic strength.

Salad dressings are acidic oil-in-water emulsions prepared with vegetable oil, i.e., mayonnaise and its imitators, egg yolk, and optional ingredients such as salt, stabilizers, thickeners, citric acid, and malic acid. Salad dressings differ from mayonnaise in that they also contain starch paste as thickener and may

contain polysaccharide gums or cellulose derivatives. Salad dressing standards require not less than 30% vegetable oil nor less than 4% liquid egg yolk (7). Egg yolk is commonly employed in food emulsions to decrease the oil/water interfacial tension (8). The proportion of oil and egg should be balanced to obtain the desired body, viscosity, and texture. The major component is generally the continuous phase, and the minor component the dispersed one. In mayonnaise, the major phase, oil, is the dispersed phase. The more oil that is dispersed, the stiffer will be the emulsion (7).

One possible application of modified SPI may be as ingredients in low-cholesterol salad products with properties similar to salad dressings or mayonnaise. Egg yolk lipoproteins prevent premature coalescence of emulsions; modified SPI could replace these lipoproteins to achieve the same result.

Rheological properties of mayonnaise and salad dressings have been studied, especially their viscoelastic properties such as elastic and loss moduli (9), and flow and consistency indexes (8,10,11). However, there are still many aspects to explore regarding rheological properties of noncommercial emulsions such as those prepared with modified SPI.

In a previous work, dynamic properties of aqueous dispersions of modified SPI were determined by varying storage and loss moduli with protein concentration and heating. Flow properties of dispersions were estimated through apparent viscosity, and flow and consistency index measurements (6).

SPI modified by acid-thermal treatment, more suitable from the rheological point of view, were selected to study their emulsifying properties. Thus, the influence of the nature of the isolates on the rheological properties of the resulting emulsions was analyzed. The effects of protein concentration and thermal treatment on rheological properties, such as apparent viscosity, flow and consistency indexes, and elastic moduli of their oil-in-water emulsions, were determined.

MATERIALS AND METHODS

Preparation of acid- and thermal-treated SPI. SPI were prepared from defatted flour provided by Santista Alimentos (Porto Alegre, Brazil). Alkali (pH 8) solubilization was conducted for 2 h at 20°C, followed by centrifuging at 6,000 × g for 30 min at 10°C. Proteins in the supernatant were precipitated at the isoelectric point (pH 4.5) and centrifuged at 5,000 × g for 15 min at 20°C. The isoelectric precipitate was resuspended in water at a 5% w/w protein concentration. The resulting

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dispersion was adjusted to pH 1.6 and heated to 90°C for 30 min to obtain the TH1.6 isolate. A fraction of this dispersion was neutralized to pH 7 to obtain the TH1.6N isolate. The pH was adjusted to the desired values with 0.5 M HCl or NaOH. Finally, the dispersions were frozen and freeze-dried (6).

Protein contents of soy isolates TH1.6 and TH1.6N were determined by the Kjeldahl method ($N \times 6.25$) to be 78.55 ± 1.99 and $72.78 \pm 0.155\%$ (g protein/100 g isolate), respectively.

Preparation of soy protein emulsions. TH1.6 and TH1.6N were used at different protein concentrations (6, 8, and 10% w/w) to formulate soy protein emulsions. Oil-in-water emulsions were prepared by homogenizing the isolate with NaCl (1% w/w), refined corn oil (Molinos Rio de La Plata S.A., Buenos Aires, Argentina) (36% w/w) and water for 1 min at 7,800 rpm at room temperature in an Ultra-Turrax T25 (dispersing tool S25N-10G; IKA-Labor Technik, Staufen, Germany) (12). An aliquot of each emulsion was heated at 90°C for 30 min and cooled first for 10 min in a water bath at 15°C and then for 48 h at 4°C.

Commercial controls. A commercial salad dressing (SD) (Hellman's, Refinerías de Maiz S.A., Buenos Aires, Argentina) and mayonnaise (MY) (Ri-k brand, Molinos Rio de La Plata S.A.) were used as controls.

Emulsion flow properties. Torque (S) was measured at 20°C in a Haake Rotavisco RV2 viscometer (Karlsruhe, Germany) with an NV sensor system (a concentric cylindrical system for measuring viscosity). This system consisted 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 for 1 min at maximum speed. Rheograms (shear stress vs. shear rate) for all samples were obtained. Apparent viscosity (η_{app}) of emulsions was calculated at the maximum shear rate (692 s^{-1}) according to the following equation:

$$\eta_{app} = \frac{GS}{n} \quad [1]$$

where G is the instrument factor in cP/degree of scale \times min, S is the degree of scale, and n is the rotor velocity in rpm (13). After converting S to shear stress (τ) and n to shear rate ($\dot{\gamma}$), the Ostwald–De Waele model was applied to the rheograms, over a shear rate of 346 s^{-1} , to calculate the flow (m) and consistency (K):

$$\tau = K(\dot{\gamma})^m \quad [2]$$

Emulsion viscoelasticities. Tests were conducted in a Haake CV20 rheometer with an NV rotor using a 1-mm gap parallel-plate sensor. Emulsions were placed on the lower plate, which was maintained at 20°C. The equipment was driven through the Haake software osc. 2.0. The linear viscoelasticity range of the dispersions was determined by measuring the complex modulus (G^*) as a function of deformation ($f = 1 \text{ Hz}$). Experimental data were obtained by recording the storage (G') and loss (G'') moduli as a function of oscillation frequency within the linear viscoelasticity range ($d = 8\%$). The dynamic behavior (G' vs. frequency) and $\tan \delta$ (G''/G') values of emulsions were studied before and after the thermal treatment.

Statistical analyses. Each measurement was carried out in duplicate. Data were analyzed by ANOVA using Systat software (Systat, Inc., Evanston, IL). Differences among the results of several treatments were studied by the Tukey test at $P < 0.05$. Differences between means ($\Delta_{0.05}$) for each rheological parameter were calculated (6).

RESULTS AND DISCUSSION

Emulsion flow behavior. Rheograms obtained for emulsions prepared with the modified SPI are shown in Figure 1. Acid isolate TH1.6 produced pseudoplastic emulsions with slight thixotropic behavior, except for those prepared with the lowest protein concentration (6%). An increase in protein concentration from 8 to 10% increased shear force with no change in thixotropy (Fig. 1A). Heating the isolate at 90°C for 30 min increased thixotropy in those emulsions with higher protein concentration. This result was more evident using 10% protein (Fig. 1B). Emulsions obtained with isolate TH1.6N showed pseudoplastic behavior with no thixotropy and higher shear stress than those formulated with TH1.6. At 10% protein and maximum shear rate (692 s^{-1}), the shear force of the former was twofold higher (Fig. 1C). The absence of thixotropy in these emulsions indicated that the flocculation phenomenon of isolates is reversible; therefore, the shear-thinning effect observed in concentrated emulsions may be due to a flocculation \rightleftharpoons deflocculation reaction (an equilibrium process), which depends on shear rate (14).

Thermal treatment did not affect the rheological behaviors of emulsions prepared with TH1.6N at 6 and 8%, whereas those prepared with TH1.6N at 10% under the same conditions showed a significant increase in shear stress. An important difference between the ascending and descending portions of the rheograms was observed (Fig. 1D). A synergistic effect between heating and protein concentration was responsible for the flow properties observed for these emulsions. Emulsions prepared with TH1.6N at 10% protein showed plastic-like flow behavior after thermal treatment (15). In general, these systems became permanently deformed when submitted to shear forces exceeding their elasticity thresholds, corresponding to viscoplastic behavior. The system starts to flow when the cohesion force among the particles is overcome. In particular, this behavior is represented by the horizontal portion of the curve (Fig. 1D) (15).

Shear force values (shear rate = 692 s^{-1}) for the unheated emulsions were statistically similar to those of the SD, whereas those of heated emulsions were similar to those of the MY (Fig. 2).

Proteins in TH1.6 and TH1.6N were totally denatured (6). Neutralization after denaturation led in TH1.6N to protein aggregation favoring protein–protein interactions (6). Consequently, plastic-behaving emulsions with high hydrodynamic volumes were generated.

Emulsion apparent viscosity, consistency, and fluidity. η_{app} for all TH1.6N emulsions were significantly higher ($P \leq 0.05$) than those prepared with TH1.6 at the same protein concentrations. In almost all cases, η_{app} increased slightly after heating,

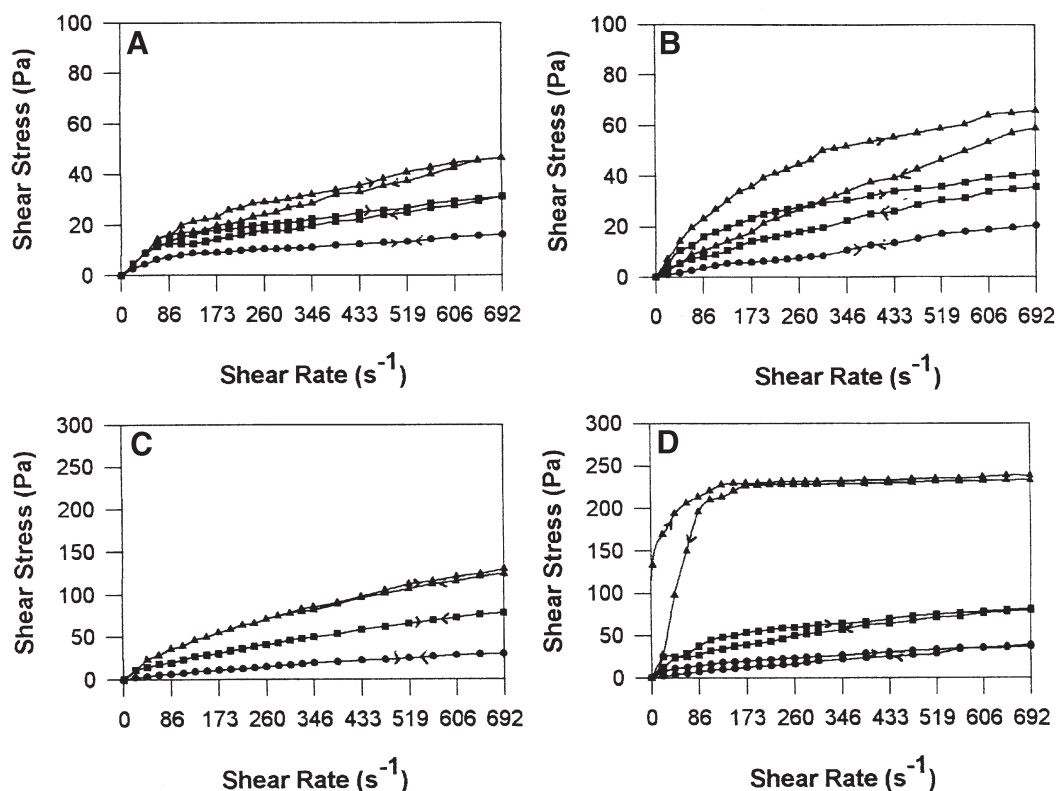


FIG. 1. Rheograms (shear stress vs. shear rate) of modified soybean protein emulsions: TH1.6 (A, B), TH1.6N (C, D). Thermal treatments: nonheated samples (A, C); heated (90°C for 30 min) samples (B, D). Protein concentration (w/w): (●) 6, (■) 8, and 10% (▲). TH1.6, soy protein isolate subjected to pH 1.6; TH1.6N, soy protein isolate subjected to pH 1.6, then neutralized to pH 7.

except for TH1.6N at 10%. For these emulsions, the thermal treatment increased η_{app} to ≈ 320 mPa·s (Fig. 3).

Stabilization of the emulsions by protein polymers is relevant in almost all food applications. Protein polymers have two effects: They can gel the continuous phase at high protein concentrations through protein cross-linkings, and they provide strong repulsive forces when adsorbed at the oil/water interface (16).

Flow index values (m) were less than 1 (Fig. 4), indicating that the emulsions were pseudoplastic fluids (7). Unheated TH1.6 emulsions did not show significant differences ($P \leq 0.05$) in consistency or in fluidity when the protein concentration increased (Figs. 4A,B). After heating, the most consistent emulsions were obtained at 10% protein (Figs. 4A,B). Unheated TH1.6N emulsions had the same flow behavior as TH1.6 emulsions (Figs. 4C,D). However, the thermal treatment applied to TH1.6N emulsions with 10% protein significantly increased their consistencies and decreased their fluidities (Figs. 4C,D). These results were directly related to the structural modifications of the isolates because of the thermal treatment. Increased temperature enhanced solvent-macromolecular segment interactions, favoring unfolding (17). Heating at 90°C for 30 min produced isolates with low solubility and high water imbibing capacity (WIC) due to protein denaturation and high surface hydrophobicity (6). This behavior was more evident in TH1.6N isolates in which salt addition during neutral-

ization caused the aggregation of high-molecular-mass proteins. Consequently, the resulting emulsion became more structured. Besides the possibility of replacing egg yolk and starch, soybean proteins could serve as water-soluble emulsifiers.

Dynamic properties of dispersions and emulsions. Figure 5 depicts the variation in the elastic moduli of emulsions with

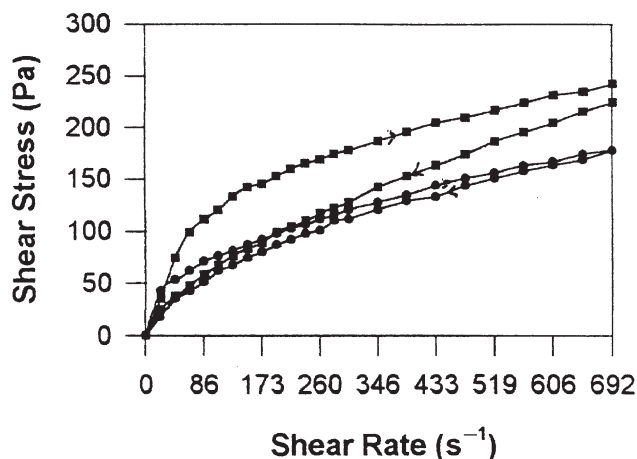


FIG. 2. Rheograms (shear stress vs. shear rate) of commercial samples: (●) salad dressing, (■) mayonnaise.

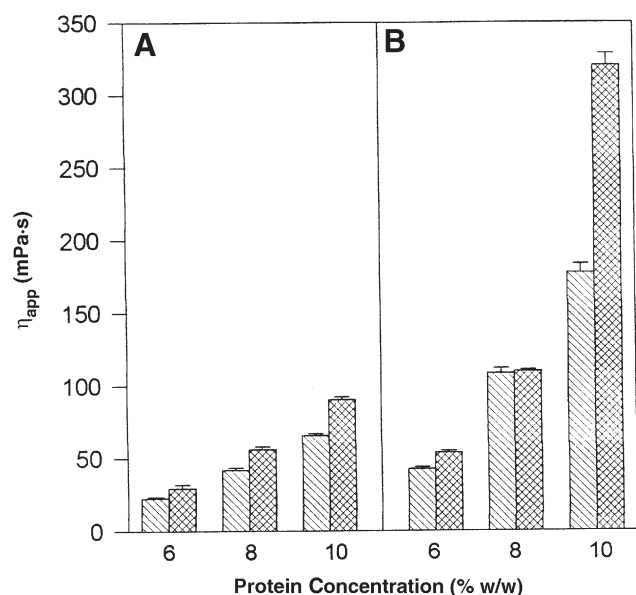


FIG. 3. Apparent viscosity (η_{app}) of TH1.6 (A) and TH1.6N (B) emulsions. Thermal treatment: diagonally lined blocks, unheated; cross-hatched blocks, heated (90°C for 30 min). $\Delta_{0.05} = 10.38$ = differences between means at 95% confidence interval.

respect to frequency. Unheated TH1.6 and TH1.6N emulsions developed low G' values (8–10 Pa at $f = 1$ Hz). These values were almost the same at all protein concentrations for TH1.6 (Fig. 5A) and slightly increased in 10% TH1.6N emulsions (Fig. 5B). Thermal treatment of emulsions led to an important increase of G' at 6% protein concentration (Figs. 5C,D). At a protein concentration of 10%, TH1.6N emulsions showed a large increase in G' moduli compared to TH1.6 samples. This solid-like structure was more elastic than those of the SD and MY (Fig. 6). In most of the emulsions, similar behavior of the loss moduli (G'') compared to the elastic moduli (G') was observed, so that the viscoelasticities of the samples were similar (Table 1). In the case of the TH1.6N-10% emulsion, the elasticity significantly increased with the thermal treatment (Table 1).

Comparison of rheological properties of dispersions and emulsions. In previous work, the rheological properties of aqueous dispersions prepared with TH1.6 and TH1.6N isolates were studied (6). The flow properties of unheated TH1.6 emulsions (10%) were similar to those of the corresponding aqueous dispersions, whereas those prepared with TH1.6N (10%) showed a notable decrease in η_{app} . With respect to dispersions, the emulsions dropped in consistency and increased in fluidity (Table 2). TH1.6 and TH1.6N dispersions behaved as nonpseudoplastics, for which the Ostwald–De Waele model cannot be applied to determine the m and K indices. Unheated emulsions were more fluid and had lower consistency than the control samples (Table 2). In the same way, protein emulsions had significantly lower η_{app} than the commercial products.

Heated TH1.6 emulsions (10%) gave flow and consistency indices ($m = 0.40$, $K = 6$) close to those obtained for the SD ($m = 0.54$, $K = 5.3$), whereas heated TH1.6N emulsions (10%) gave flow index values significantly lower ($m = 0.05$) and con-

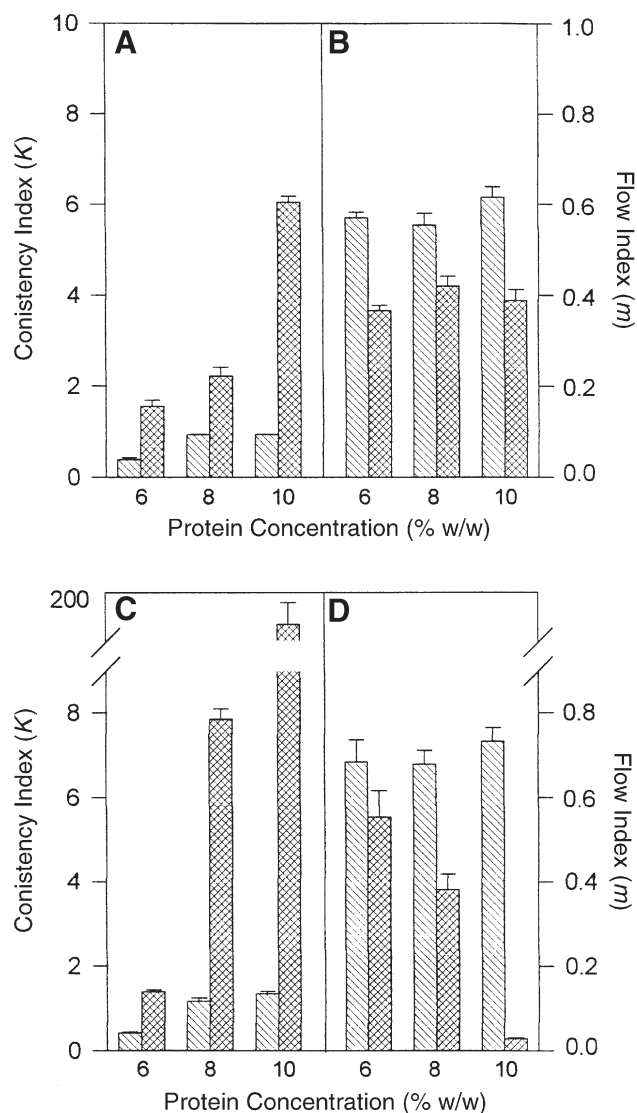


FIG. 4. Consistency index (A, C) and flow index (B, D) of TH1.6 (A, B) and TH1.6N (C, D) emulsions. Thermal treatment: diagonally lined blocks, unheated; cross-hatched blocks, heated (90°C for 30 min). $\Delta_{0.05 (m)} = 0.381$, $\Delta_{0.05 (K)} = 0.094$. For abbreviations see Figures 1 and 3.

sistency indices ($K = 193$) considerably higher than those for the more consistent commercial MY ($m = 0.20$, $K = 65.8$).

Table 3 shows the elastic moduli of both emulsions and dispersions, the later being reproduced from Puppo and co-workers (6). From these results, it is evident that oil supplementation to TH1.6 dispersions reduced the elastic component of the resulting emulsion, even after thermal treatment. The effect was exactly the opposite for TH1.6N isolates, suggesting that the structural difference of the isolate proteins permitted the development of emulsions with different viscoelastic properties than those of the corresponding dispersions. Since the elastic modulus (G') represents the recoverable energy when a material is subjected to deformation, the increase in the elastic modulus indicates a more solid-like emulsion with a more complex structure (18,19). The elastic moduli of TH1.6N

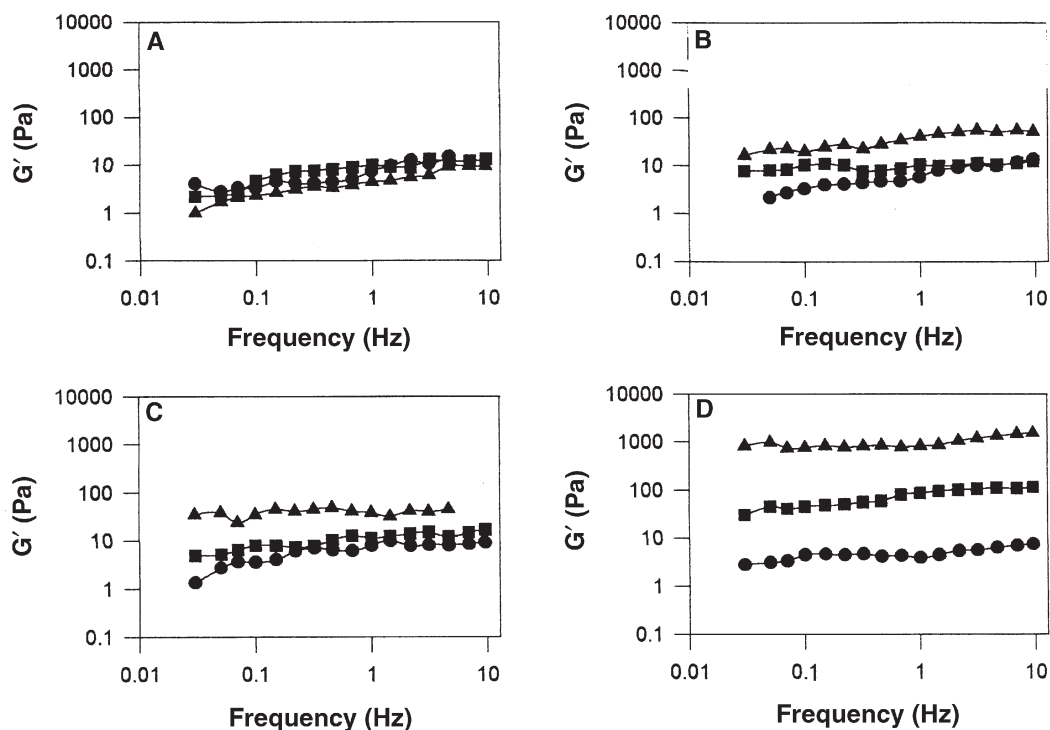


FIG. 5. Dynamic oscillatory rheological analysis. Frequency dependence of storage modulus, G' , of TH1.6 (A, B) and TH1.6N (C, D) emulsions. Thermal treatment: nonheated samples (A, C); heated (90°C for 30 min) samples (B, D). Protein concentrations (w/w): (●) 6, (■) 8, and 10% (▲).

emulsions increased after adding oil; therefore, we assumed that the interaction between the isolate microgel and the emulsion droplets was responsible for the viscoelastic behavior of TH1.6N emulsions. Protein aggregation at neutral pH could be established through noncovalent bonds and/or S-H/S-S interchange. At high concentrations, protein molecules could form a viscoelastic structure that stabilizes the emulsion. A similar effect was observed by Ma and Barbosa-Cánovas (20) with xanthan gum in emulsions. This solid-like structure was more elastic than those of SD and MY, as we deduced from Figures 5 and 6.

SPI was chosen as the sole emulsifier and gelling agent for this work because it is known that protein-covered oil droplets act as active fillers, enhancing the strength of emulsion gels (21,22). In a previous work (23), the emulsifying properties of these isolates were studied. The results are shown in Table 4. Isolate TH1.6 had a higher emulsifying capacity and higher tendency to coalesce than TH1.6N. Both isolates had the same stability with regard to flocculation-creaming. Kiosseoglou and Sherman (24) proposed that although protein adsorption is a relatively slow process, flocculation proceeds rapidly with droplet coalescence as the dominant process. This process leads to constant reduction in viscoelasticity. Similarly, TH1.6, which forms emulsions with less elasticity, apparent viscosity, and consistency, gave the lowest stability with respect to coalescence. The lower emulsifying capacity of TH1.6N resulted from the high aggregation degree of the isolate proteins. This

structure prevents unfolding but leads to the formation of a film with low coalescent capacity, generating emulsions of high consistencies and stabilities.

Ford and co-workers (25) demonstrated that aggregation could be used to formulate dressings. An aggregated system has a higher viscosity than a nonaggregated system. Thus, it is possible to formulate a dressing with less oil and/or less gums and stabilizers regulating aggregation.

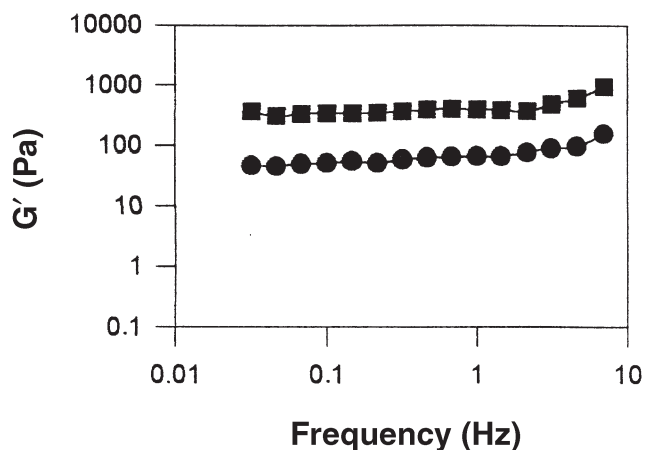


FIG. 6. Frequency dependence of storage modulus, G' , of commercial samples: (●) salad dressing, and (■) mayonnaise.

TABLE 1
Viscoelasticities of Emulsions Measured as G''/G' Ratio^a (tan δ)

tan δ	TH1.6			TH1.6N		
	6%	8%	10%	6%	8%	10%
Unheated	1.01 \pm 0.42	1.20 \pm 0.49	1.24 \pm 0.41	0.90 \pm 0.41	1.04 \pm 0.33	1.48 \pm 0.36
Heated ^b	1.33 \pm 0.32	0.69 \pm 0.22	1.48 \pm 0.18	1.40 \pm 0.34	1.21 \pm 0.26	0.78 \pm 0.12

^a G' , storage modulus; G'' , loss modulus; TH1.6, soy protein isolate subjected to pH 1.6; TH1.6N, soy protein isolate subjected to pH 1.7, then neutralized to pH 7.

^bAt 90°C for 30 min.

TABLE 2
Flow Parameters of Nonthermally Treated 10% w/w Dispersions and Emulsions of TH1.6 and TH1.6N Isolates and Commercial Samples of Salad Dressing (SD) and Mayonnaise (MY)

Sample	TH1.6-D	TH1.6-E	TH1.6N-D	TH1.6N-E	SD	MY
η_{app} (mPa·s)	57.6 \pm 0.0	65.7 \pm 1.3	34.8 \pm 1.2	177.3 \pm 6.4	250.5 \pm 14.8	296.2 \pm 8.8
K (mPa·s)	2.7 \pm 0.2	0.94 \pm 0.0	6.4 \pm 3.8	1.34 \pm 0.06	5.3 \pm 0.3	65.8 \pm 3.8
m	0.42 \pm 0.01	0.62 \pm 0.23	0.22 \pm 0.09	0.73 \pm 0.32	0.54 \pm 0.02	0.20 \pm 0.01

^aD, dispersion; E, emulsion; η_{app} , apparent viscosity; K , consistency; m , flow; for other abbreviations see Table 1.

TABLE 3
Elastic Moduli (G') of 10% w/w Dispersions and Emulsions of TH1.6 and TH1.6N Isolates, SD and MY^a

Sample	TH1.6-D	TH1.6-E	TH1.6N-D	TH1.6N-E	SD	MY
G' (Pa) ^b	113.0 \pm 4.8	3.5 \pm 1.4	10.3 \pm 3.3	43.5 \pm 2.1	63.6 \pm 2.9	374.5 \pm 7.8
G' (Pa) ^c	185.0 \pm 24.0	41.7 \pm 5.3	266.0 \pm 14.0	711.0 \pm 154.1	—	—

^aFor abbreviations see Tables 1 and 2.

^bSamples not heated.

^cSamples heated at 90°C for 30 min.

TABLE 4
Emulsifying Properties of TH1.6 and TH1.6N Isolates^a (from Ref. 23)

	Emulsifying capacity (g oil/g isolate)	Emulsion stability (%)	Coalescence (%)
TH1.6	5818	45	75
TH1.6N	2800	44	17

^aFor abbreviations see Table 1.

ACKNOWLEDGMENTS

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

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[Received July 6, 2001; accepted January 20, 2003]