Functional Properties of Soy Protein Isolates As Affected by Heat Treatment During Isoelectric Precipitation

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Research was carried out on the influence of heat treatment during isoelectric precipitation on the functionality of the resulting isolates prepared from "low-heated," undenatured, defatted soy flour. The isoelectric dispersions were heated at 50°C, 60°C, 65°C or 70°C for 30 min and characterized for solubility, water absorption capacity, oil absorption capacity, viscosity and gel properties. The effect of heat treatment during isoelectric precipitation was reflected by all the functional properties. The soy isolate heated at 60°C showed remarkably different flow characteristics. It yielded the most viscous dispersions and the strongest gels and also the highest water absorption capacity value. Simple and multiple lineal regression models were used to provide a description of the relationship between the different properties studied. Significant correlations were found between gel viscosity and water absorption capacity. Gel characteristics also correlated significantly with viscosity of 20% dispersions. Viscosity of dispersions correlated to solubility or to water absorption capacity, depending on protein concentration. That dependence was quantified and a highly significant multiple correlation was found between viscosity and the independent variables of log solubility and water absorption capacity.

KEY WORDS: Functional properties, isolation, processing effect, soybean proteins.

Oilseeds constitute an important source of proteins for human consumption. However, to be exploited successfully, those proteins must be presented in forms that are attractive and possess the flavor, texture and quality desired by the consumer. The properties that determine the behavior of proteins in foods during processing, storage and consumption are collectively called functional properties (1). During the past thirty years there has been an increasing interest in the use of soy protein products in both traditional and processed foods (2). Soy protein isolates are used as an ingredient in dairy analogs and in meat-based products such as processed meats, sausages, meatballs, hams and pork, where they perform several useful functions, *e.g.* moisture and fat holding, gelling, emulsifying, structure formation and adhesion.

The successful use of soy proteins depends on the versatility of their functional properties. Those properties are influenced by intrinsic factors (composition and conformation of proteins), environmental factors (composition of the model system or food) and by the methods and conditions of isolation (3). Commercial soy protein isolates are prepared from minimum heat-treated soy flour by dissolving the protein in diluted alkali, removing the insoluble materials and precipitating the protein at pH 4.5. Usually the isoelectric curd is neutralized and spray-dried.

Since different processing treatments affect functionality, it is important to study the effect of individual steps during protein recovery processes and then to relate different processing technologies with specific functional characteristics. The objective of this work was to investigate the influence of heat treatment during isoelectric precipitation on functionalities of soy protein isolates.

EXPERIMENTAL PROCEDURES

Preparation of soy protein isolates. Soybean protein isolates were prepared from 1985-crop, "low-heat" undenatured defatted soy flour by dispersing soybean flour in distilled water (1:10, w/w), adjusting pH to 8 with 10% NaOH and mixing at 250 rpm at room temperature for 1 hr. The mixtures were then centrifuged at 1,232 *g* for 30 min, and the supernatants were adjusted to pH 4.5 with diluted HCl. The isoelectric dispersions were heated in a water bath at 50°C, 60°C, 65°C or 70°C for 30 min after reaching the specified temperatures. Dispersions were then centrifuged at 1,232 *g*, and the isoelectric curds were washed twice with water (1:5, w/w). The isolated protein was neutralized to pH 7.0 with 10% NaOH and spray-dried in a Niro Atomizer 53 (Niro Atomizer Ltd. Copenhagen, DK-2860 Soeborg-Denmark) spray drier. Drying conditions, in terms of inlet and outlet temperatures were 240°C and 110°C, respectively. The Na-proteinates had a particle size of 90 μm. Table 1 shows the moisture, protein (N × 6.25) and ash content of soybean flour and heat-treated protein isolates.

Analytical methods. Moisture and ash were determined according to AACC (4). Nitrogen was determined by Kjeldahl method (5).

Nitrogen solubility was measured according to AOCS NSI method (6). Water absorption capacity (WAC), expressed as mL water/g protein, was determined at 20°C in the device designed by Torgensen and Toledo (7). Oil

TABLE 1

Crude Protein (% N × 6.25) and Ash Content of Soybean Flour and Isolates

Samples	Crude protein (%, db)	Ash (%, db)
Soybean flour, 30 min	51.06	5.51
Isolate-treated at 50°C, 30 min	93.76	2.80
Isolate-treated at 60°C, 30 min	92.75	3.25
Isolate-treated at 65°C, 30 min	93.77	3.18
Isolate-treated at 70°C, 30 min	93.94	3.16

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absorption capacity (OAC), expressed as mL oil/g protein, was determined according to Kanterewicz *et al.* (8). For viscosity measurements, soy proteins (10%, 14% and 20% dispersions, w/w) were dispersed in distilled water at 11,000 rpm in a Virtis "45" homogenizer (The Virtis Company, Inc. Gardiner, New York, NY) for 2 min. The proteins were allowed to swell for 20 min, and the dispersions were remixed for 2 min. Viscosities were determined at 20°C with a cone-and-plate Brookfield LVT viscometer (Brookfield Engineering Laboratories, Stoughton, MA) with stainless steel cones Cp 40, Cp 41 and Cp 52. The measurements were carried out at 12 rpm. Gels were made by heating 20% slurries of protein isolates in sealed containers at 80°C for 1 hr. The containers were cooled overnight at 4°C and gel viscosities were measured at 20°C on a Brookfield RVT viscometer (Brookfield Engineering Laboratories) by using the Helipath stand (Brookfield Engineering Laboratories) with the T-spindle series at 0.5 rpm. Water binding properties of gels were determined by the net-test developed by Hermansson and Lucisano (9). Moisture loss was calculated as (weight of juice released/weight of sample) × 100.

Statistical analysis. All analyses were performed in duplicate. Simple and multiple lineal regression models were used to provide for a description of the relationship between the various functional properties measured. It was carried out by using an IBM PC.

RESULTS AND DISCUSSION

Functional properties. The effect of heat treatment on isoelectric dispersions was reflected on all functional properties. As shown in Figure 1, solubility decreased as temperature increased, probably due to denaturation of soy protein. This loss of solubility was more pronounced above 60°C. Denaturation studies of soy proteins by differential scanning calorimetry showed that the denaturation process of soy protein dispersions at pH 4-5 starts above 60°C (10).

Water absorption capacity showed a completely different behavior (Fig. 2). It increased drastically as temperature of the treatment increased from 50°C to 60°C and dropped slightly for higher temperatures. These results are in agreement with those reported by Johnson (2) and López de Ogara *et al.* (11). As nitrogen solubility index (NSI) decreases, water absorption increases to a point and then decreases again with diminishing solubility. Maximum WAC values correspond to solubilities in the range of 60% to 70%. The oil absorbing capacity increased slightly by heating (Fig. 2). As protein denaturation progresses, as shown by a decrease in solubility, hydrophobicity usually increases due to the gradual exposure of hydrophobic residues, which are often buried in the interior of the native protein molecule, as a result of protein unfolding (12). Figure 3 shows the viscosity behavior of soy protein dispersions for all the protein concentrations tested. Viscometric characteristics are influenced by both solubility and water absorption. The relative importance of these properties will depend on the quantitative relationship between total and imbibed water. This relationship is termed water ratio (T/I) and is expressed as g total water/g water imbibed by the protein (13). When T/I is above 1, free water exists that drastically reduces internal friction between the solid particles, and consequently viscosity is lowered. At a T/I of 1 or below, all the water in the system is imbibed water. This causes great internal friction and high viscosity. Figure 4 shows that the isolate heated at 60°C for 30 min exhibited the lowest T/I values, while the isolates that yielded less viscous suspensions exhibited higher T/I values for all the concentrations tested. Figure 5 shows the characteristics of protein gels. Viscosity increased as temperature of treatment increased from 50°C to 60°C and then dropped for higher temperatures. Moisture loss, as expected, showed the opposite behavior (11). Consequently, the 60°C isolate produced the strongest gel with high viscosity and low

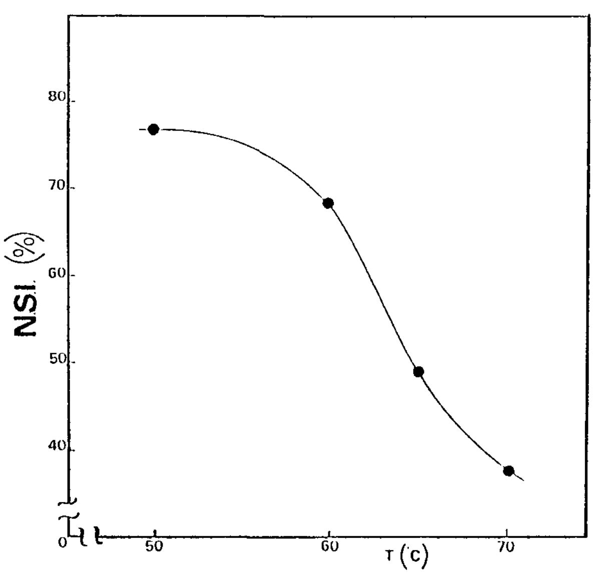

FIG. 1. Solubility of soy protein dispersions as a function of temperature.

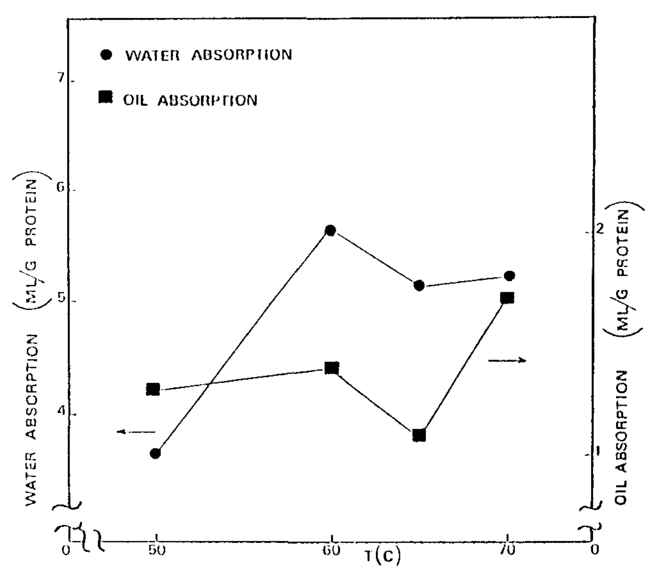

FIG. 2. Effect of temperature on the water and oil absorption capacity of soy protein isolates.

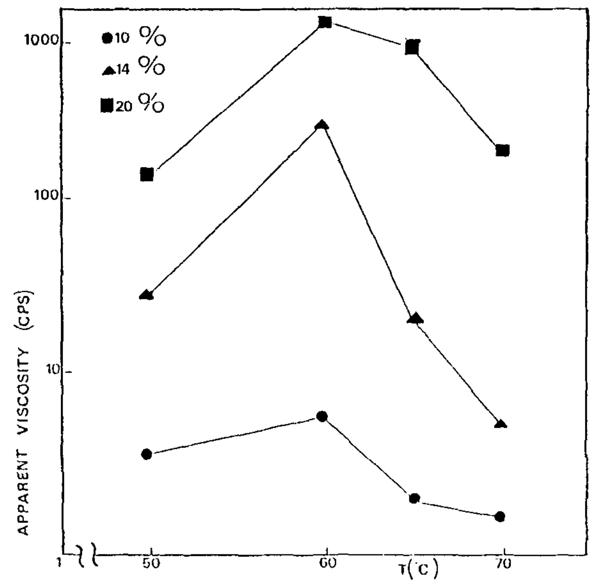

FIG. 3. Effect of temperature on the apparent viscosity of 10%, 14% and 20% dispersions of soy protein isolates.

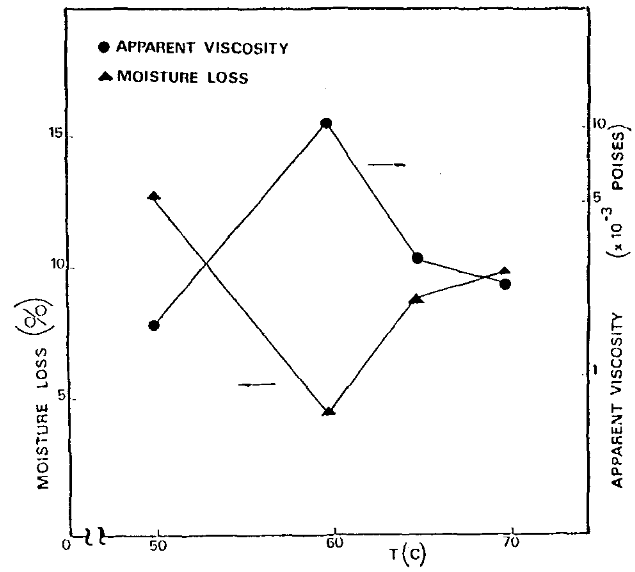

FIG. 5. Effect of temperature on the apparent viscosity and moisture loss from gel prepared from 20% protein concentration.

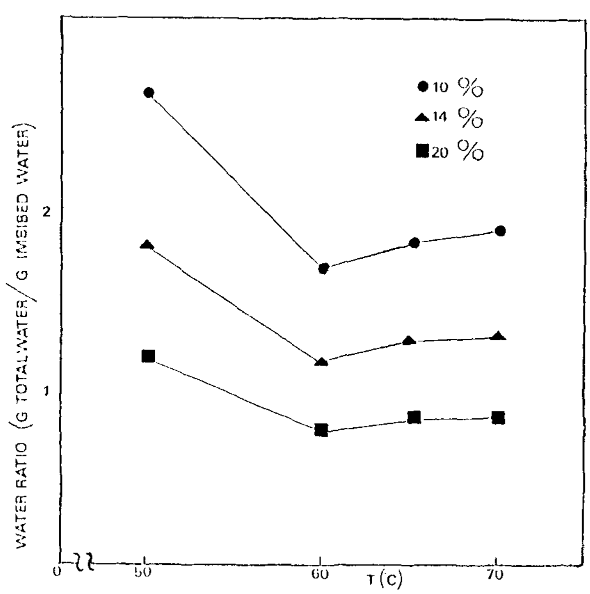

FIG. 4. Water ratio of 10%, 14% and 20% dispersions of soy protein isolates as a function of temperature.

moisture loss. Gel formation is a process involving several different reactions (denaturation, association, dissociation and aggregation), which requires extremely specific conditions. Gelation properties are complexly interrelated to solubility, WAC and viscosity (14). From Figures 2 and 3, WAC and viscosity of 20% dispersions also showed a maximum at 60°C. On the other hand, isolated soy proteins with a NSI of 80% or more do not possess the gelling properties of proteins with an NSI in the range of 50% to 60% (2). Under the conditions of the test, the solubility and flow properties of the 60°C isolate were suitable for optimal protein gelation.

TABLE 2

Correlation Coefficients Between Functional Properties of Soybean Proteins[a]

Function	Viscosity of protein dispersions			Gel moisture loss
	10%	14%	20%	
Solubility	0.858 (1)	*	*	*
Water absorption	*	*	0.862 (1)	*
Gel moisture loss	*	*	−0.888 (1)	1.000
Gel viscosity	*	0.817 (5)	0.841 (1)	0.954 (1)

[a]Numbers in parentheses indicate level of significance.
*No significant correlation coefficient (at 1% or 5% level).

Relationship between functional properties of soybean proteins. Table 2 shows only the significant simple correlation coefficients between the various functional properties studied. As expected, a significant correlation was found between the two gel properties studied (8). Gel characteristics also correlated significantly with viscosity of 20% dispersions. For the lowest concentration tested (10%), viscosity of dispersions was significantly correlated with solubility, while it correlated with WAC for the higher concentration (20%). To quantify this dependence, a multiple lineal regression model was used. The best regression model included WAC and log solubility as independent variables for the three concentrations studied. Examination of the coefficients of determination (R^2) and T values (Table 3) indicated that WAC and solubility were largely responsible for the viscosity behavior. Results showed that for the 20% dispersion, 81.7% of the variability in viscosity could be accounted for by the two independent variables. For 14% and 10% dispersions, these percentages were still higher (99.9% and 95.4%, respectively). The β-coefficients (standardized regression coefficients) suggested that the relative importance of solubility and WAC in determining the viscosity behavior depended on

TABLE 3

Multiple Regression Models for Prediction of Viscosity Behavior of Soy Protein Dispersions As Influenced by Protein Concentration

Dependent variable	Variable description	R^2 (n = 5)	Regression coefficient	β-value	T-value
Viscosity of 10% protein dispersions	Constant		−3.706		
	Log solubility	0.954[a]	2.014	1.095	10.22[b]
	WAC		0.134	0.475	4.43[a]
Viscosity of 14% protein dispersions	Constant		−11.273		
	Log solubility	0.999[a]	5.635	1.082	140.41[b]
	WAC		0.599	0.752	97.51[b]
Viscosity of 20% protein dispersions	Constant		−3.028		
	Log solubility	0.817[c]	1.795	0.544	2.53[c]
	WAC		0.509	1.009	4.69[a]

[a]$P<0.01$. [b]$P<0.001$. [c]$P<0.05$.

protein concentration. For 20% dispersions, the β value for WAC doubled that for log solubility. For the lowest concentration, this ratio was inverted while for the intermediate concentration, both WAC and log solubility were of equal importance. This indicates that, at high protein concentrations, where all the water in the system is imbibed by the protein, viscosity is governed by WAC. In more diluted dispersions, where free water exists, viscosity is primarily governed by solubility.

The results suggest that the protein isolate heated at 60°C showed particularly noteworthy flow characteristics. It also possessed the highest WAC and a good solubility and could be the most suitable to be used in meat-based products, where those properties are of prime importance.

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