Protein Solubility Characteristics of Commercial Soy Protein Products

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ABSTRACT: Solubility characteristics of commercial soy protein products (flours, concentrates, and isolates) were determined under various conditions. From the solubility profiles at various pH values and NaCl concentrations, soy protein isolates can be divided into three groups. One group had high solubility near the pI. Another group had low solubility near the pI, but high solubility at pH 11. The third group had low solubility even at pH 11. Except for the hydrolyzed products, the protein solubilities of the soy protein products at various salt concentrations were very low. Temperature did not significantly affect the protein solubility, although a few products showed more than a 20% increase at temperatures >50°C. Soy protein concentrates and soy protein isolates showed similar solubility profiles. The proteins in all commercial products (except flours) tested were denatured, as evident from the solubility profiles in the presence of salt and the enthalpy values from DSC.

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KEY WORDS: Commercial soy products, concentrates, flours, isolates, protein, protein solubility, soybeans, soy protein.

The manner in which proteins behave in a food system, i.e., their functionality, depends on the fundamental physical and chemical properties of the proteins under given conditions (1). Much research regarding the relationship between physicochemical properties and functional properties of food proteins has been conducted (2–4). Furthermore, physicochemical properties of food systems generally are sensitive to past processing history, methods of preparation, and conditions of measurements. Understanding the physicochemical states and interactions of the protein is necessary to predict functional behavior of the protein products. However, many reports have dealt with pure proteins, such as protein isolates, 11S protein (glycinin), and 7S protein (conglycinin), that were prepared carefully in the laboratory. Little has been reported on physicochemical and functional properties using commercial protein products (5,6).

Knowing the solubility profiles of soy protein products in various environmental conditions is important to the industry in evaluating other physicochemical and functional properties in order to screen them for potential applications. Solubilities of soy protein products are highly dependent on the physicochemical states of protein molecules, which are either favorably or adversely affected by heating, drying, and other processing treatments during their manufacture and storage (5). This property is therefore one of the most widely used characteristics of protein products. A better understanding of the solubility characteristics of soy protein products can be obtained over a wide range of environmental conditions including pH, ionic strength, and temperature.

The objective of this research was to characterize commercially available soy protein products for protein solubility. This information can be used as an important part of a comprehensive database that both industrial and academic researchers can use in identifying and selecting potential starting materials for their product development work.

EXPERIMENTAL PROCEDURES

Materials. Commercial soy protein products were obtained from various manufacturers. Protein isolates were from Archer Daniels Midland Co. (ADM, Decatur, IL), and Protein Technologies International, Inc. (PTI, St. Louis, MO). Protein concentrates were from Central Soya Co., Inc. (Fort Wayne, IN) and ADM. Flours were from Cargill Protein Products (Cedar Rapids, IA). The isolates were coded as I, concentrates as C, and flours as F. The protein content of each product is reported on an as-is basis (Table 1).

Protein solubility. Protein solubility was measured by using the method of Morr *et al.* (7) with minor modifications. The sample (about 500 mg) was dispersed into 50 mL of 0.1 M NaCl solution. After adjusting the pH with 1 N NaOH or 1 N HCl solution, the sample suspension was thoroughly mixed using a shaking water bath at 25°C. The pH of suspension was monitored and adjusted again after 1 h, and yet again after 30 min of shaking. The suspension was then centrifuged at 27,000 × *g* for 30 min followed by filtering through Whatman No. 41 filter paper. The protein content of the filtrate was measured by using the Kjeldahl method and the conversion factor 6.25. The ionic strength (μ) of salt solutions was calculated using the equation, $\mu = {}^1/_2 \Sigma c_i Z_i^2$, where c_i and Z_i are the molar concentration and the electrical charge of *I* component, respectively. The ionic strength of the NaCl solution was the molarity itself, but that of the $CaCl_2$ solution was ${}^1/_3$ of its molarity. The effects of temperature on protein solubility were determined by shaking the protein solution at 50 and 75°C in 0.1 M NaCl at pH 7. The error range between duplicates was within 2%.

DSC. The modified method of Arnfield and Murray (8) was used to prepare the sample and take measurements. Slurries of

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TABLE 1
Codes of Soy Protein Products[a] and Their Protein Contents[b]

Code	Product brand name	Protein content (wt%)	Code	Product brand name	Protein content (wt%)
I-1	Profam 974	86.12 ± 0.47	C-1	PromaxPlus	62.05 ± 0.04
I-2	Profam 981	87.80 ± 0.32	C-2	Promine DS	64.95 ± 0.19
I-3	H200FG	83.25 ± 0.13	C-3	Promine HV	63.49 ± 0.12
I-4	Ardex DHV, dispersible	89.41 ± 0.05	C-4	Promax 70	60.80 ± 0.18
			C-5	Promax 70L	63.91 ± 0.20
I-5	Ardex D	87.25 ± 0.37	C-6	Promine VPH	60.92 ± 0.21
I-6	Ardex D, dispersible	86.18 ± 0.41	C-7	Procon 2000	64.54 ± 0.51
			C-8	Procon 20/60	63.23 ± 0.11
I-7	Ardex F	87.20 ± 0.11	C-9	Cenpro 70	58.33 ± 0.01
I-8	Ardex F, dispersible	87.20 ± 0.26	C-10	Arcon G	62.27 ± 0.11
			C-11	Arcon S	66.04 ± 0.01
I-9	Suproplus 2600	34.42 ± 0.36	C-12	Arcon F	63.17 ± 0.36
			F-1	100/90	52.03 ± 0.17
I-10	Suproplus 2100	54.91 ± 0.05	F-2	200/70	51.88 ± 0.25
			F-3	200/70 + 6%	48.02 ± 0.19
I-11	Suproplus 675	78.31 ± 0.23	F-4	200/70 + 15%	44.35 ± 0.05
I-12		75.81 ± 0.55	F-5	200/20	52.26 ± 0.02
I-13	Procote 200	80.00 ± 0.08	F-6	200/20 + 6%	48.74 ± 0.28
I-14	Procote 400	81.46 ± 0.04	F-7	200/20 + 15%	43.98 ± 0.03
I-15	Procote 5000	87.67 ± 0.44			
I-16	Supro 710	87.86 ± 0.40			
I-17	Supro 660	85.39 ± 0.14			
I-18	Supro 500E	87.07 ± 0.30			
I-19	Supro HD-90 FP 920	87.17 ± 0.18			

[a]Products, I-1 through I-8 and C-10 through C-12 were from Archer Daniels Midland Co. (Decatur, IL); I-9 through I-19 were from Protein Technologies International, Inc. (St. Louis, MO); C-1 through C-9 were from Central Soya Co., Inc. (Fort Wayne, IN); and F-1 through F-7 were from Cargill (Cedar Rapids, IA).
[b]Moisture contents ranged from 5 to 8%.

protein products (20% w/w) were prepared in distilled water by rod-mixing in an Eppendorf tube (1 mL) for 1 min. The sample (35–45 mg) was placed in a preweighed aluminum DSC sample pan, hermetically sealed, and weighed to 0.001 mg accuracy. A PerkinElmer DSC-4 was used to measure thermal properties. Temperature calibration and the calibration coefficient (K) for the DSC cell were determined using indium standard over a scanning range of 50–200°C. All samples were scanned at 10°C/min heating rate in the range of 50–115°C using instrument sensitivity range of 1 mcal/s. An empty sample pan was used as reference, and the measuring cell was flushed with nitrogen gas at 20 psi for all runs. A Bravender recorder (Model 3021-21, 10 mV) was used. To calculate the enthalpy of reaction, the following equation was used:

$$\Delta H = 60 \cdot K \cdot A \cdot R / W \cdot S \quad [1]$$

where ΔH = energy (mcal/mg), K = calibration coefficient (cm^{-1}), A = peak area (cm^2), R = range (mcal/s), W = sample weight (mg), and S = chart speed (cm/min). Peak area A was determined from the correlation curve of area against chart weight. Duplicate measurements were done. As a control, native soy protein was isolated from the soy flour (Sigma) at pH 7 by using the general isoelectric precipitation method (5).

RESULTS AND DISCUSSION

Effect of pH. The conditions used for determining protein solubility vary substantially between methods. The official method requires stirring without pH adjustment and centrifuging at 1,500 rpm to remove insoluble particles. This method, however, has a critical drawback—insufficient removal of insoluble particles that hinder filtering the supernatant after centrifuging. The solubility difference in soy protein is small between the low and high centrifugation speed conditions. The solubility difference was as low as 10% between the official method and the method used in this report. Protein solubilities of protein isolates at various pH values are shown in Figure 1. The protein solubility changes with pH were similar for isolates I-1 through I-8, with only I-7 showing lower solubility than the others. Some isolates (I-9 through I-19) exhibited three distinctly different trends in protein solubility. The first group had high solubility (>30%) at all pHs tested. The second group had low solubility (<5%) at pH 4.3 but became highly soluble at alkaline pH levels, similar to the typical solubility profile of native soy protein. The last group had low solubility (<40%) at pH 11. One isolate product (I-14), however, was completely soluble at pH >7. The sharp differences in protein solubility among isolate products suggest that their protein subunit

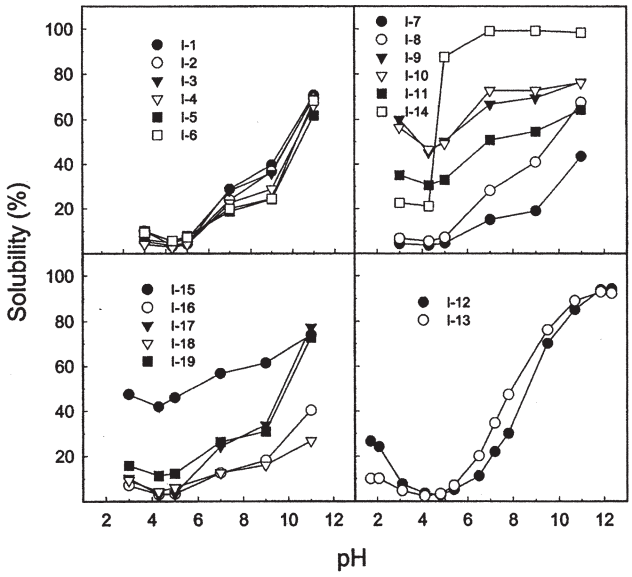

FIG. 1. Effect of pH on protein solubility of isolate products in 0.1 M NaCl at 25°C. For product codes and protein content, see Table 1.

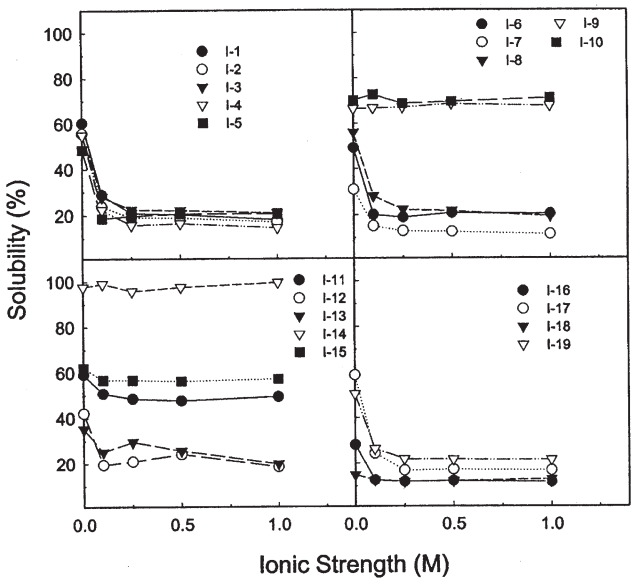

FIG. 3. Effect of ionic strength (Na^+) on protein solubility of isolate products at pH 7, 25°C. For product codes and protein content, see Table 1.

compositions might have been different. Protein isolates in the high protein solubility group contained more low-M.W. subunits than those in the low protein solubility group based on SDS-PAGE analysis (data not shown). Protein conformation may also be different depending on the degree of protein denaturation. The initial pH in 0.1 M NaCl was as low as pH 4.6 for I-12 and I-13 isolates. However, protein solubility followed the general pattern after pH adjustment and stabilization to the set values.

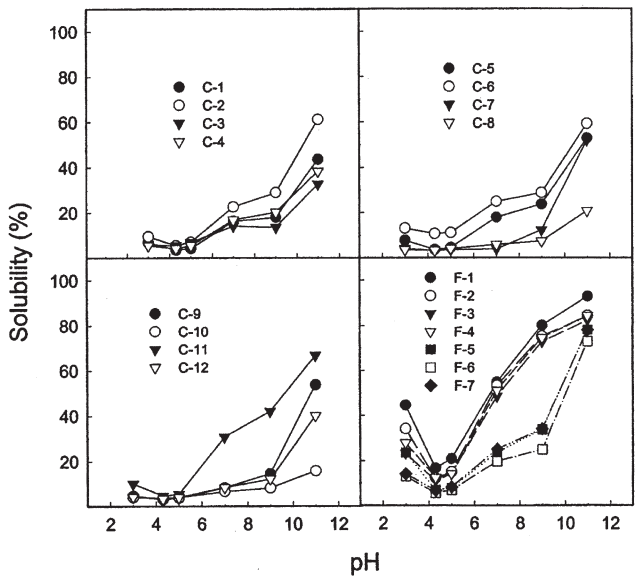

FIG. 2. Effect of pH on protein solubility of concentrate and flour products in 0.1 M NaCl at 25°C. For product codes and protein content, see Table 1.

Figure 2 shows the protein solubility profiles of the concentrates and flours. Protein solubilities of C-8 and C-10 were lower than for the other products over the entire pH range due to their large particle size. F-1 and F-2 products had higher protein solubilities than F-5. The lecithin contents of the soy flours did not affect solubilities as shown in relecithinated products. In general, solubility did not increase as sharply as for native soy protein when pH was increased, except for F-1 and F-2. The low-solubility group of protein isolates and concentrates had lower solubility than the low-solubility flour products. This suggested that significant protein damage may have occurred during processing.

Effects of monovalent ions. NaCl was used as the source of monovalent ions. Figure 3 shows the ionic strength effects on the protein solubilities of isolates. Structure-stabilizing effects of salt decrease protein solubility. This salting-out phenomenon was evident when there was a small increase in salt concentration, after which it leveled off. Three distinct trends were also observed in protein solubility-pH profiles: A high-solubility group maintained high solubility at all salt concentrations tested; the next group showed decreased solubility with increasing salt concentration; and the third, low-solubility, group maintained low solubility regardless of the salt concentration. Almost all isolate products had typical solubility profiles of denatured soy proteins (5). Figure 4 shows the ionic strength effects on the solubilities of concentrates and flours. All protein concentrates were similar to isolates and exhibited the denatured soy protein pattern. F-1 and F-2 products had the native soy protein profile. Solubility decreased at 0.1 M salt concentration, and then increased with increasing salt concentration until the original value was attained at 1.0 M. The solubility profiles exhibited steep salting-out phenomena at low salt concentrations until solubility minima were reached at 0.1 and 0.2

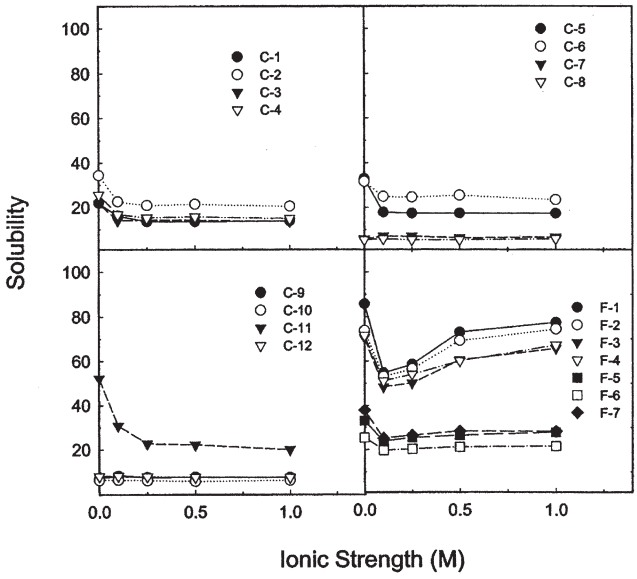

FIG. 4. Effect of ionic strength (Na^+) on protein solubility of concentrate and flour products at pH 7, 25°C. For product codes and protein content, see Table 1.

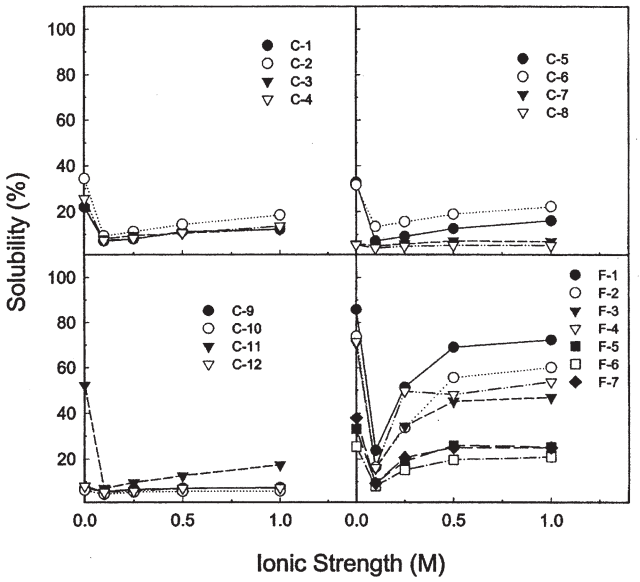

FIG. 6. Effect of ionic strength (Ca^{2+}) on protein solubility of concentrate and flour products at pH 7, 25°C. For product codes and protein content, see Table 1.

M of monovalent salt concentrations for the native and denatured soy protein isolates, respectively. For a given salt, denatured soy protein isolates were, on the average, salted out at twice the rate of native soy protein isolates (9). The protein isolates in the high-solubility group did not significantly change regardless of salt concentration.

Effects of divalent ions. Figure 5 shows solubility profiles of isolates at various $CaCl_2$ concentrations. A solubility minimum occurred at 0.1 M ionic strength, equivalent to 0.033 M $CaCl_2$, and the solubility increased with the salt concentration. Many products still maintained high protein solubility, while the solubilities of a few others decreased with increasing Ca^{2+} concentrations. Small-M.W. protein would be the major protein in these products. Figure 6 shows solubility profiles of soy protein concentrates and soy flours at various Ca^{2+} concentrations. The protein solubility of all concentrate products tended to depend on Ca^{2+} concentrations. Flour products were different from the concentrate products, in that the minimal solubility (F-1 and F-2 products) was reached at 0.1 M ionic strength, followed by a steep increase in protein solubility with increasing Ca^{2+} concentration. Hermansson (10) reported the effect of $CaCl_2$ on the solubility of an isolate prepared under mild conditions. At low Ca^{2+} concentrations, protein solubility sharply declined at all alkaline pHs. It was postulated that binding of Ca^{2+} might have a bridging effect that also reduced the net negative charge on protein molecules. Protein solubility increased at higher concentrations of $CaCl_2$, so solubility became pH independent at 0.2 M $CaCl_2$ concentration. Salts exert two distinctive effects on the protein solubility: an electrostatic shielding effect at concentrations below 0.2 M and an ion-specific effect at concentrations above 0.2 M. The ion-specific effect of $CaCl_2$ salt was not evident in this study, further suggesting that the protein conformation in these products is denatured.

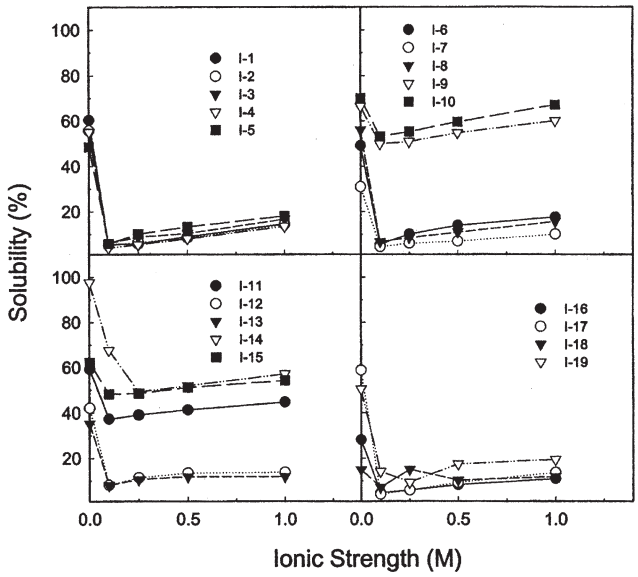

FIG. 5. Effect of ionic strength (Ca^{2+}) on protein solubility of isolate products at pH 7, 25°C. For product codes and protein content, see Table 1.

Effects of temperature. The study of the effect of temperature on protein solubility was carried out at 25, 50, and 75°C, at pH 7.0, and in 0.1 M NaCl. The effects of temperature on protein solubility were small for most of the isolates (Fig. 7). However, protein solubility increased by more than 20% for some products (I-12 and I-13) at 50°C compared to that at 25°C. Soy protein is known to be a stable protein to heat, more

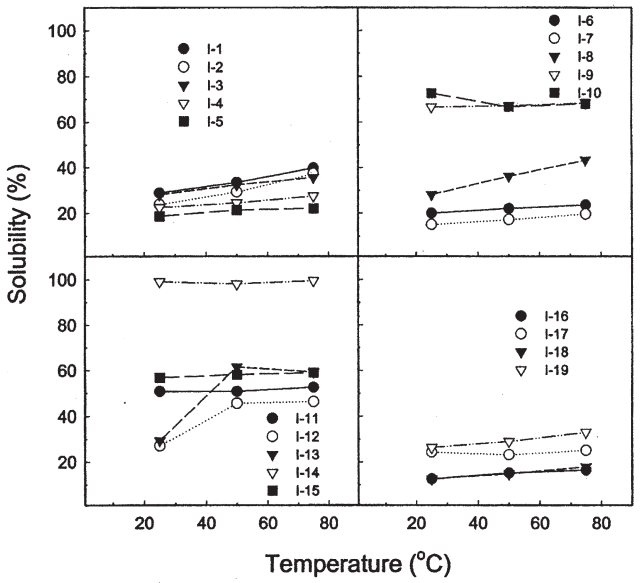

FIG. 7. Effect of temperature on protein solubility of isolate products at 0.1 M NaCl, pH 7. For product codes and protein content, see Table 1.

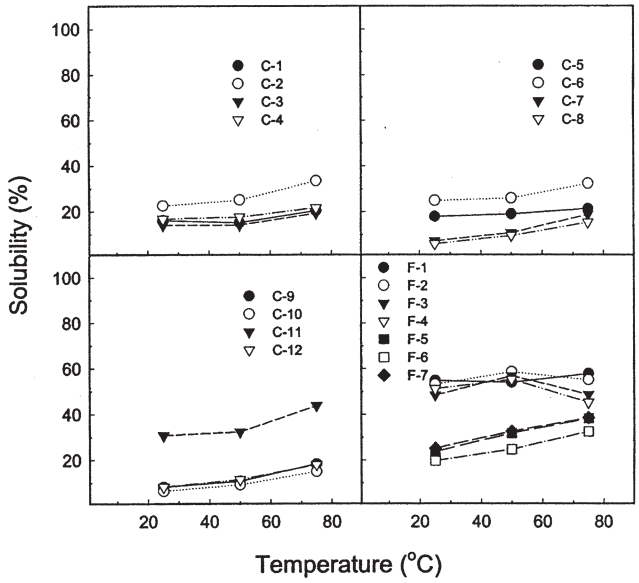

FIG. 8. Effect of temperature on protein solubility of concentrate and flour products at 0.1 M NaCl, pH 7. For product codes and protein content, see Table 1.

so than animal proteins. They maintain their solubilities to 70–80°C. The effects of temperature on protein solubility of concentrates and flours are shown in Figure 8.

Thermal analysis. Endothermic peaks in DSC thermograms originate from the two major proteins (7S and 11S proteins) of the soy protein, and the peak area (enthalpy) of the protein product correlates with the degree of protein denaturation (8). Preliminary results on a native soy protein isolate, prepared under mild conditions (30°C, pH 7.6), showed the first endothermic peak for 7S protein at t_{max} = 78.5°C, and the second endothermic peak from 11S protein at t_{max} = 97.5°C. Enthalpy of the native soy protein was calculated to be 2.18 cal/g protein by the area sum of both peaks when isolated under mild laboratory conditions. The smaller enthalpy value of the native soy protein found in this study as compared to the published one, 3.4 cal/g protein (11), seemed to originate from the low sensitivity of the instrument and the preparation method of the sample. Enthalpies of the protein products are shown in Table 2. The large SD of enthalpy values reported in this table may have resulted from the difference in instrument sensitivity and methods used for sample preparation. Care was taken to suspend samples homogeneously in water during the sample preparation. The isolates and concentrates did not show endothermic peaks except in I-5 and I-6 products, whose enthalpies were 0.73 and 0.84 cal/g protein, respectively. Hermansson (12) reported thermograms of a few commercial soy protein isolates in which endothermic peaks were absent. Flours showed endothermic peaks as expected; however, enthalpy values for F-5 to F-7 products were lower than F-1 to F-4 products. These results support previous data on protein solubility in the presence of salt that showed that almost all the proteins in isolates and concentrates were denatured.

TABLE 2
Degree of Protein Denaturation of Soy Protein Products as Determined by the DSC Method

	Enthalpy		Degree of protein denaturation
Product	cal/g product	cal/g protein	(%)
I-5	0.64 ± 0.18	0.73 ± 0.18	66.5
I-6	0.72 ± 0.11	0.84 ± 0.11	61.5
I-1–I-19 except I-5, I-6	0	0	100
C-1–C-12	0	0	100
F-1	1.04 ± 0.03	1.99 ± 0.03	8.7
F-2	1.11 ± 0.10	2.14 ± 0.10	1.8
F-3	0.82 ± 0.10	1.72 ± 0.10	22.1
F-4	0.89 ± 0.08	2.01 ± 0.08	7.8
F-5	0.65 ± 0.10	1.24 ± 0.10	43.1
F-6	0.67 ± 0.10	1.37 ± 0.10	37.2
F-7	0.63 ± 0.10	1.43 ± 0.10	34.4

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