Effects of Steaming on Soybean Proteins and Trypsin Inhibitors

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Steaming of defatted soybean flakes decreases both protein solubility and trypsin inhibitor (TI) activity as measured by nitrogen solubility index (NSI) and TI assay, respectively. NSI can be lowered from 80–90, in raw flakes, to 10–20 with sufficient steaming. Concurrently, about 90% of TI activity is destroyed. Differential scanning calorimetric (DSC) analysis of raw soy flour demonstrated transition temperatures (T_{max}) of 58.2, 72.8 and 96.4°C for TI, 7S and 11S proteins, respectively. Lowering the NSI to 42.4 by steaming caused the TI transition to disappear, even though half of the TI activity remained. DSC analysis of steamed soybean flakes revealed that undenatured 7S and 11S storage proteins remain, even when the NSI is decreased to 10.

KEY WORDS: Differential scanning calorimetry, nitrogen solubility, NSI, protein, soybean, soy flour, steaming, trypsin inhibitor.

Soybean proteins in their various commercial forms, such as defatted flours, concentrates and isolates, offer high nutritional value (1,2) and desirable functionality (3). As a result, soybean protein products are included in a wide variety of formulated foods. Antinutritional factors, such as trypsin inhibitors (TI), are present in raw soybeans but the effects of these are destroyed for the most part by a moist heat treatment referred to as toasting (4–7). Improvement in nutritional value as a result of toasting is attributed to both lowered TI activity and increased digestibility of the soy storage proteins (8). Solubility of soy flour proteins is used as a measure of the extent of this heat treatment (9).

Differential scanning calorimetry (DSC) has proven to be a useful technique for observing and measuring change-of-state transitions that materials may undergo as a result of a change in conditions (10). Isolated and purified soybean storage proteins have been studied by this technique (11–16). Thermal denaturation transitions (T_{max}) were reported to occur between 68–84 and 84–108°C for 7S and 11S proteins, respectively. However, the physical characteristics of isolated proteins may not be the same as the proteins *in situ*. DSC makes it possible to study materials in their original milieu. The soybean storage proteins, 7S and 11S, and the Kunitz and Bowman-Birk TI's are collected into protein bodies (17–19). Opportunities for interactions and reactions among these proteins should be enhanced as a result of their close proximity in protein bodies. For this reason, physical treatments and chemical reactions performed on the proteins of soybean flour might lead to unexpected results and unique properties not observed when the same treatments or reactions are carried out with isolated soy proteins.

Therefore, reported here are the results of DSC experiments with raw and toasted soy flours. Data are also presented on the TI levels and protein solubilities of these soy flours.

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MATERIALS AND METHODS

Samples. Commercial raw soybean flakes, which had received only mild heat treatment to remove residual hexane after oil extraction (Nutrisoy 7B), were obtained from the Archer Daniels Midland Co., Decatur, IL. Certified seed-grade soybeans, Hack and Century varieties, were purchased from the Kelly Seed Co., Peoria, IL. Whole soybeans at 8–10% moisture were cracked by passage through continuous milling rolls and dehulled. After tempering to 12–14% moisture, the cracked dehulled beans were flaked at ambient temperature by passage through smooth rolls; average flake thickness was 0.254 mm. Flakes were defatted by batch extraction (1.6 mL solvent/g flakes) eight times at ambient temperature with pentane-hexane (boiling range 33–57°C). Residual pentane-hexane was removed in a hood by passing ambient air over the sample for 64 h. Portions of the three soybean flake samples were subjected to live steam in an autoclave at atmospheric pressure (100°C) for various times, air-dried and ground into flour. The raw and heat-treated flakes were then analyzed for residual TI activity (20) and nitrogen solubility (NSI) (21).

DSC Analysis. A MicroCal MC-2 scanning calorimeter (MicroCal, Inc., Amherst, MA) was used in conjunction with the software provided by the manufacturer.

Slurries for DSC analysis were prepared by adding 1.5 mL of distilled H_2O to 0.25 g of soy flour, degassing under a vacuum and placing the degassed slurries into the DSC analytical cell. Care was taken to eliminate air pockets. Degassed, distilled H_2O was added to the reference cell. DSC scans were carried out in duplicate between 30–100°C at scan rates between 49–75°C/h.

Water-extracted soy proteins and washed residues were prepared as illustrated in Figure 1. Soy flour (1 g) was extracted with water (20 mL) by stirring for 2 h at ambient temperature. This is similar to the procedure used in the

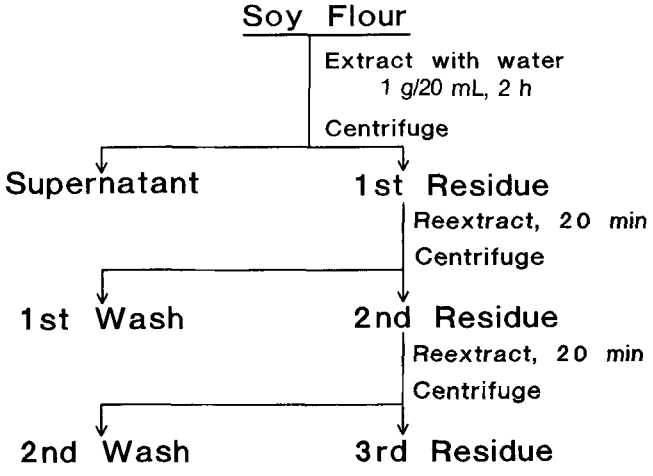

FIG. 1. Extract and residue preparation. Soy flour was water-extracted (20 mL/g) for 2 h at ambient temperature. Second and third extractions were carried out for 20 min each. Supernatants were separated by centrifugation at 34,000 relative centrifugal force for 20 min at 20°C.

NSI determination (21). After centrifugation at 34,000 relative centrifugal force (RCF) for 20 min at 20°C, the supernatant was decanted. The residue was re-extracted twice more under the same conditions. The first extract was subjected to DSC analysis as was the last washed residue.

DSC thermograms were analyzed with the DA-2 Data Ac-quisition and Analysis System supplied with the instrument. Rescans, after the initial scan on a sample, produced smooth thermograms with no transitions. Therefore, these data were stored and used for baseline corrections of the initial DSC thermograms obtained from each sample. All scan data were adjusted for the mg N in the cell.

RESULTS AND DISCUSSION

NSI and TI vs. *heating time.* Both NSI and TI decreased with increased steaming time (Fig. 2). NSI (Fig. 2a) decreased somewhat more slowly with steaming than reported by Circle and Smith (9). While they noted a decrease in NSI from 83 to 20 within 20 min, the samples steamed here required 30 min to achieve the same decrease in NSI. Similarly, the rates of TI activity decrease in Figure 2b were more gradual than those reported by Al-brecht *et al.* (22) and Rackis (5). However, considering expected variability among soybeans, the samples prepared for DSC experiments had NSI and TI results near those expected. The standard deviation (SD)

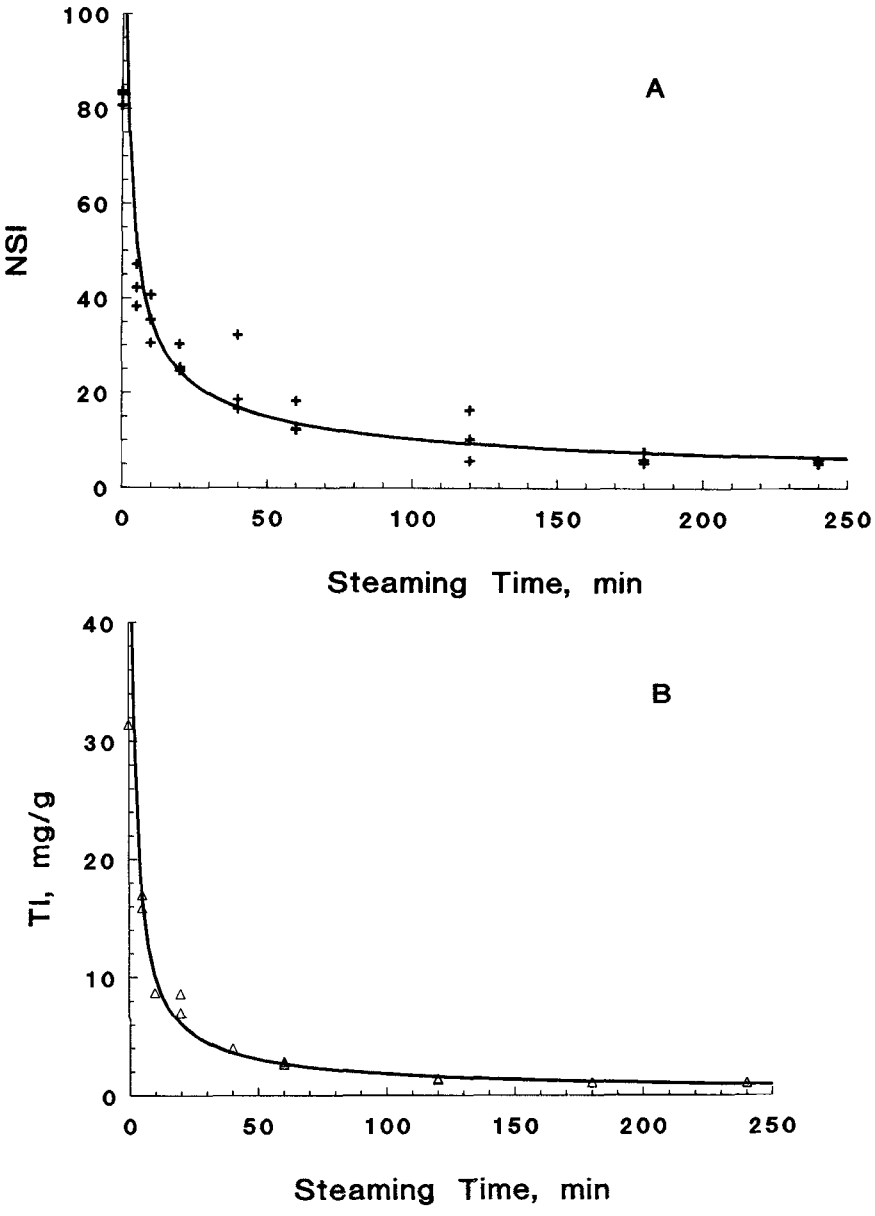

FIG. 2. Effects of steaming time on A: nitrogen solubility index (NSI) and B: trypsin inhibitor (TI) activity.

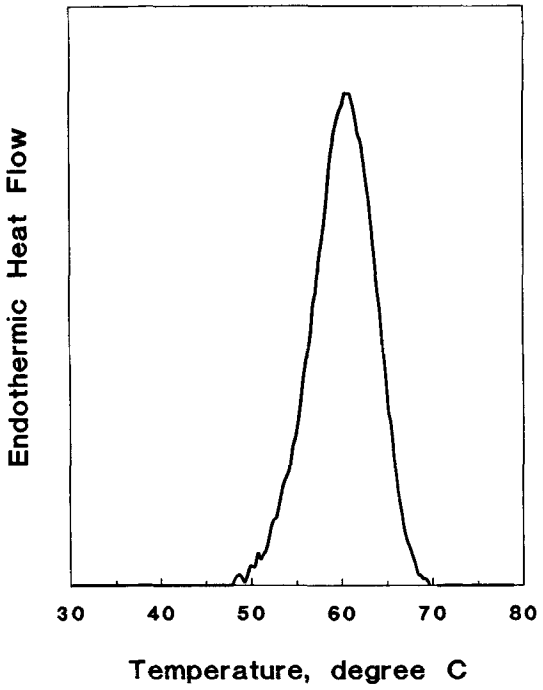

FIG. 3. Differential scanning calorimetry thermogram of Kunitz soybean trypsin inhibitor. Scan rate = 70.71°C/h.

for the NSI data was 4.36, and for the TI data SD was 0.53.

DSC of raw soy flour slurries. The DSC T_{max} for isolated Kunitz soybean TI was found to be 61.1°C (Fig. 3). For all of the scans, the 7S T_{max} occurred at 72.9°C (SD = 0.84) and the 11S T_{max} at 95.9°C (SD = 1.08). These transition temperatures were well within the range reported for isolated soy storage proteins (11–16).

DSC thermograms of the raw flours prepared in the laboratory from Century and Hack soybeans and of commercially prepared Nutrisoy 7B are shown in Figure 4. The raw flour from Century variety had an NSI of 80.7 and contained 31.4 mg TI per gram according to the standard assay (20). The thermogram for this raw flour (Fig. 4a) displays T_{max} at 57.8, 73.9 and 98.4°C for the Kunitz TI and the 7S and 11S soy storage proteins, respectively. Additionally, shoulders appear around 79.2°C on the high-temperature side of the 7S protein transition and in the area of 90.3°C on the low-temperature side of the 11S protein transition. The thermogram of raw soy flour from Hack variety soybeans (Fig. 4b) displays features similar to those observed for Century variety flour. The Kunitz TI transition is observed at 58.7°C. There are prominent transitions for both the 7S (72.2°C) and 11S (95.6°C) soy storage proteins, as well as shoulders on the high-temperature side of the 7S transition and on the low-temperature side of the 11S transition.

In contrast to the raw, laboratory-prepared, Century and Hack soy flour samples, the commercial raw flour (Nutrisoy 7B) (Fig. 4c) did not display a transition for the Kunitz TI even though all the NSI's were similar. This thermogram displayed 7S and 11S transitions at 72.3 and 95.1°C, respectively. A shoulder on the low-temperature side of the 11S transition was apparent, but a shoulder on the high-temperature side of the 7S transition was much smaller.

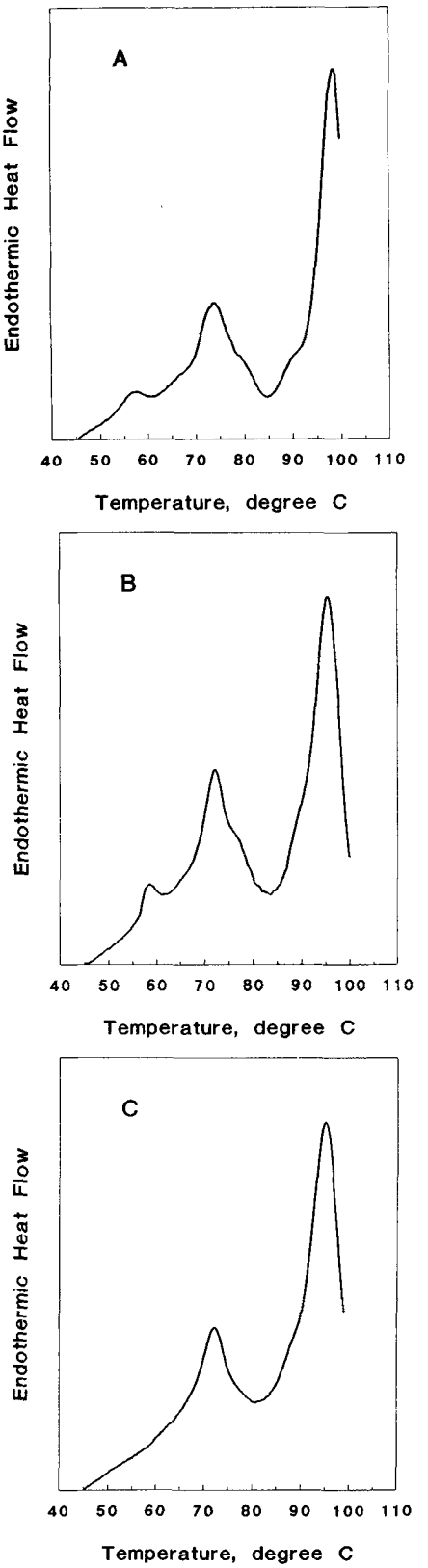

FIG. 4. Differential scanning calorimetry thermograms of raw defatted soybean flours. A: Century variety, NSI = 80.7, scan rate = 72.69°C/h; B: Hack variety, nitrogen solubility index (NSI) = 83.0, scan rate = 74.14°C/h; C: Nutrisoy 7B, NSI = 83.7, scan rate = 70.46°C/h.

DSC of toasted soy flour slurries. Steaming of Century variety soy flour, sufficient to decrease NSI to 42.4, produced a thermogram (Fig. 5a) in which the transition attributed to Kunitz TI was no longer evident. However, the standard TI assay measured 15.9 mg TI per gram which was approximately half the original activity in raw flour. The shoulders noted between the transitions for 7S and 11S in raw flour are still evident in Figure 5a. However, the minimum between these transitions was not as low as for raw flour. Presumably, the Kunitz inhibitor has interacted in some fashion with itself or with other components present in or around the protein bodies to form species undergoing transitions within this temperature range while retaining some of the inhibitor activity. The disappearance of the Kunitz inhibitor transition coupled with retention of half the inhibitor activity argues for

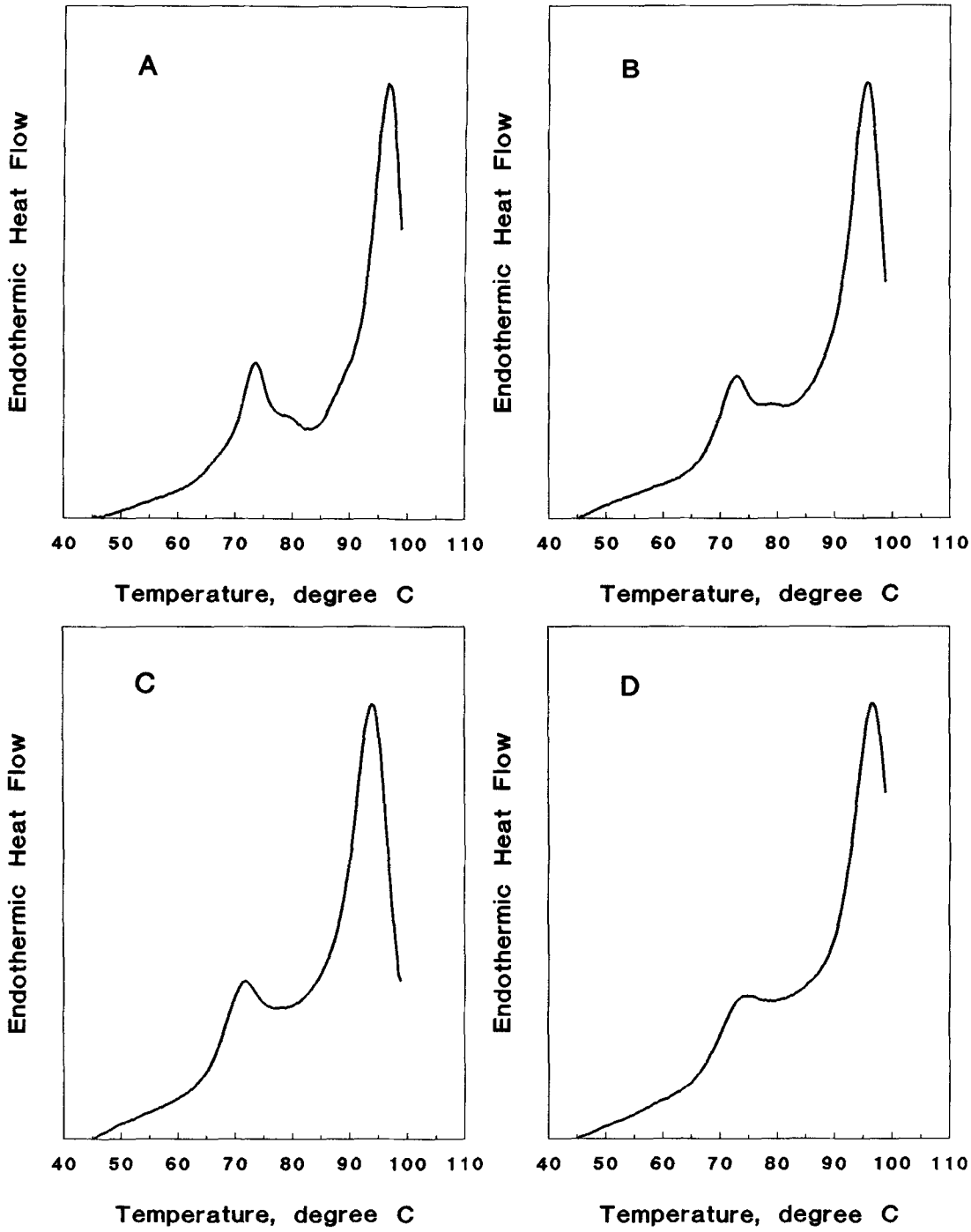

FIG. 5. Differential scanning calorimetry thermograms of laboratory-prepared steamed defatted soybean flours. Century variety A: nitrogen solubility index (NSI) = 42.4, scan rate = 72.88°C/h; B: NSI = 16.6, scan rate = 66.08°C/h; C: NSI = 12.5, scan rate = 66.50°C/h; D: NSI = 5.5, scan rate = 69.96°C/h.

some process or processes taking place other than complete and irreversible denaturation.

This trend continues with further sample treatment. Thermograms for samples with NSI's of 16.6, 12.5 and 5.5 (Figs. 5b, c and d, respectively) displayed successively less prominent minima between the storage protein transitions. The latter two thermograms displayed no discernible shoulders comparable to those seen in flours receiving less severe treatment; the area between the storage protein transitions is only a smooth shallow minimum. Most (91–99%) of the TI activity had been destroyed in these samples.

Changes in the thermograms of the commercially prepared defatted soy flour (Nutrisoy 7B) (Fig. 6) with increased steaming in our laboratory were not as apparent as with the laboratory-prepared and steamed Century variety soy flour (Fig. 5). The minimum between the 7S and 11S transitions became less prominent during the heat treatment applied to progress from NSI 83.7 (Fig. 4c) to NSI 38.3 (Fig. 6a) but changed little with further heating (Fig. 6b).

It was anticipated that thermograms of low-NSI soy flours would display no denaturation transitions for the 7S and 11S proteins. The presence of such transitions (Figs. 5 and 6) indicates that the heating received by the samples in the autoclave was qualitatively different from that in the DSC. To verify that a DSC scan from 30 to 100°C completely denatured the soy storage proteins, scanned samples were cooled in the instrument to less than 50°C and rescanned to 100°C. No transitions were observed on such rescans. Similar results were obtained with low-NSI samples from each of the three heat-treated soy flours. This demonstrates that the observed transitions were not reversible under the conditions used. Further, by the very nature of the DSC instrumentation with its feedback circuitry, when a transition is occurring, additional heat is applied to the sample cell to force completion of the transition while continuing to increase the sample cell temperature at the same rate as the reference cell temperature. Evidently, heating in the autoclave, even under harsh conditions, was insufficient to completely denature all of the storage protein.

DSC of toasted soy flour extracts and residues. Thermograms for the residues remaining after water extraction under conditions of the NSI standard procedure (Fig. 1) are shown in Figure 7. The transitions for the 7S and 11S were still prominent, even though all of the protein soluble in the NSI method was removed. The NSI for the soy flours from which these residues were prepared were 16.6 and 6.0 so that 83.4 and 94% of the protein should still have been present but rendered insoluble in water. The thermograms appeared more similar to the steamed soy flour slurries (Fig. 5) than to the raw flour slurries (Fig. 4); no evidence of a Kunitz TI transition was present, and the minimum between the 7S and 11S transitions was not as prominent as was observed in the thermograms of raw soy flour (Fig. 4).

When toasted soy flour was extracted with 0.01N NaOH, as is done in the analysis of trypsin inhibitor in the standard assay (20), a thermogram was obtained (Fig. 8) similar to those for raw soy flour slurries (Fig. 4). This included a transition in the region of the Kunitz trypsin inhibitor, although the transition was not prominent. The NSI of the soy flour from which this extract was made

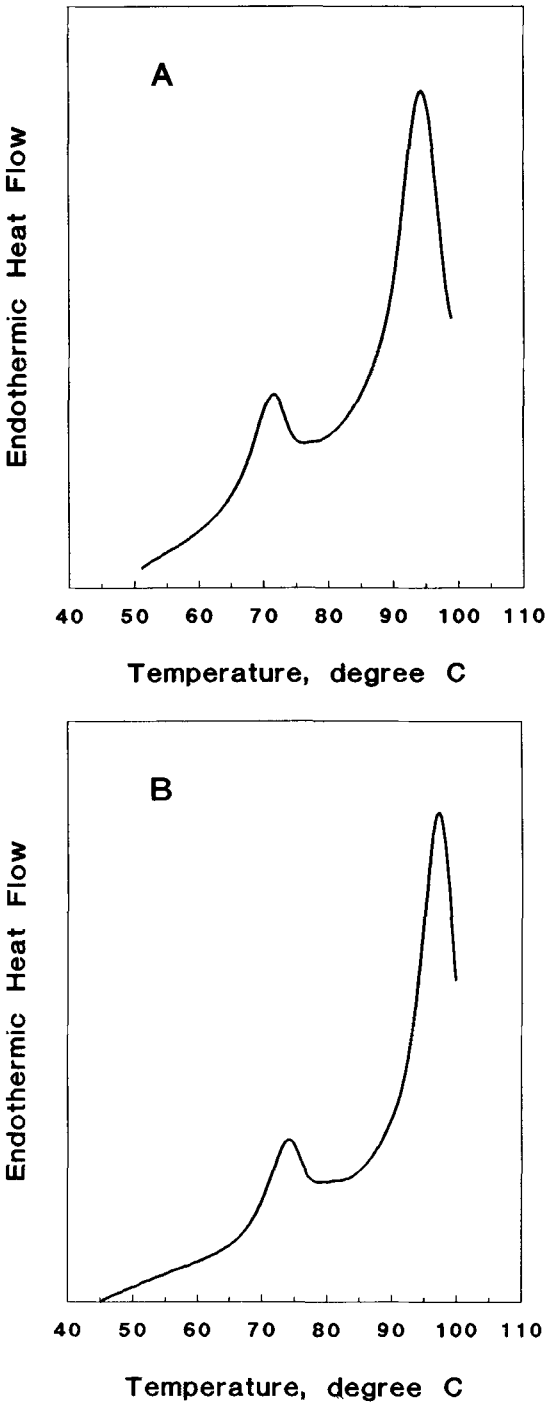

FIG. 6. Differential scanning calorimetry thermograms of steamed commercial defatted soybean flour. Nutrisoy 7B. A: nitrogen solubility index (NSI) = 38.3, scan rate = 71.26°C/h; B: NSI = 18.4, scan rate = 71.38°C/h.

was 30.6, and the TI activity was greatly diminished to 8.7 mg TI per gram soy flour. When extractions and washings similar to those depicted in Figure 1 were performed with 0.01 N NaOH, the resulting residues displayed no denaturation transitions for the 7S and 11S proteins, and the extracted protein agglomerated and precipitated when extracts were neutralized. This observation indicated that the disappearance of the Kunitz TI

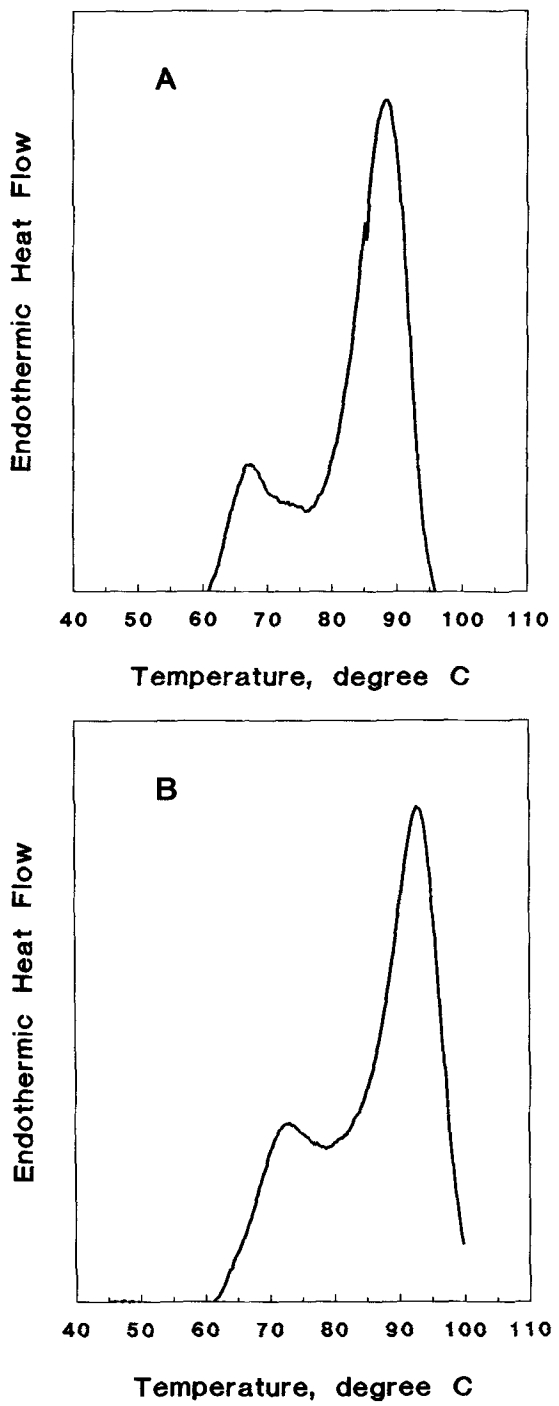

FIG. 7. Differential scanning calorimetry thermograms of water-washed residue from defatted soybean flour. Century variety A: nitrogen solubility index (NSI) = 16.6; scan rate = 56.25°C/h; B: NSI = 6.0; scan rate = 49.68°C/h.

transition, observed when raw soy flour was moist heat-treated (Figs. 4 and 5), was probably due, at least in part, to a physical interaction rather than to the formation of covalent bonds. NaOH is able to extract the inhibitor and was a main reason that alkali was introduced into the standard TI assay in place of water (23,24).

Both physical and chemical reactions appeared to take place during the steaming of soy flakes. German *et al.* (15)

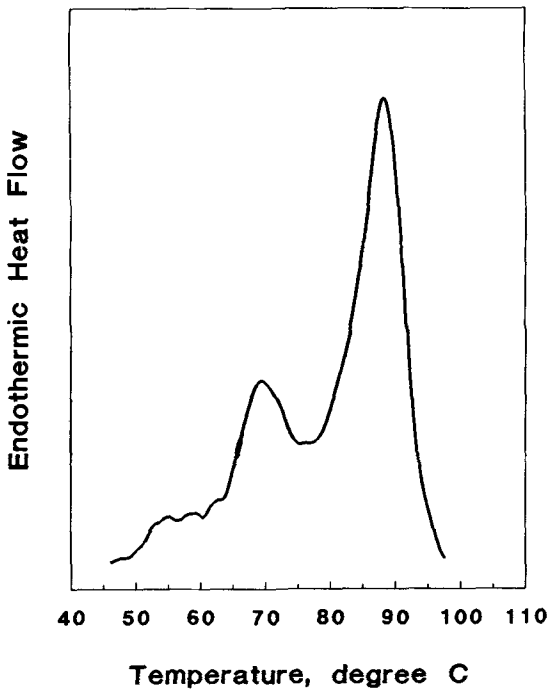

FIG. 8. Differential scanning calorimetry thermogram of alkaline extract of defatted soybean flour. Century variety. Flour nitrogen solubility index (NSI) = 30.6; scan rate = 74.90°C/h.

and Damodaran and Kinsella (25) found that heating purified 7S and 11S together resulted in complex formation, involving electrostatic interactions between dissociated 7S subunits and the basic subunits of dissociated 11S. Anderson and Hockridge (26) isolated trypsin inhibitors from toasted soy flours and subjected them to reversed-phase high-performance liquid chromatography (HPLC) analysis. New species not present in inhibitor isolates from raw flour were found among the inhibitors isolated from toasted soy flours. The amount of these new inhibitor species increased in proportion to the remaining Bowman-Birk and Kunitz inhibitors. Kim and Barbeau (27) reported the formation of soy protein aggregates upon autoclaving a soy protein concentrate. The aggregates were reported to have a molecular weight (MW) around one million daltons and it was suggested that these aggregates were held together by both noncovalent and disulfide bonds.

Marshall and Zarins (16), working with purified glycinin in 1.0% solution, found an exotherm when 2-mercaptoethanol was present in DSC experiments. This exotherm was protein concentration dependent, decreasing considerably in magnitude between 2.0 and 4.0% protein concentration. These authors presented data, which indicated that aggregation of the basic glycinin subunits was responsible for the observed exotherm, and they attributed the lack of an exotherm at higher glycinin concentrations, even though the glycinin acidic and basic subunits had been dissociated by 2-mercaptoethanol, to the spatial constraint in which the basic subunits find themselves at higher protein concentrations. Beta-conglycinin also inhibited glycinin basic subunit aggregation.

No such exothermic transition was observed in the experiments reported here. Several features of these ex-

periments would tend to prevent the aggregation of glycinin basic subunits to produce an exotherm. First, no 2-mercaptoethanol was present to reduce the disulfide bond linking acidic and basic glycinin subunits. Further, beta-conglycinin was present, although not always in undenatured form, and Damodaran and Kinsella (25) reported that beta-conglycinin inhibits the aggregation of the basic glycinin subunits through electrostatic association with the subunits. Finally, the effective glycinin concentration within protein bodies would be much higher than the 2.0–4.0% found sufficient to inhibit the formation of glycinin basic subunit aggregates. The decrease in NSI noted after steaming defatted soy flour (Fig. 2) was undoubtedly due partly to denaturation of protein leading to water insolubilization. The finding by DSC analysis of undenatured protein in extensively steamed soy flours indicated that at least some of the protein present was not extracted by water for reasons other than denaturation and insolubilization. An explanation for these results may lie in the formation during steaming of insoluble, denatured protein around the surface of protein bodies, so that undenatured protein within remains unextracted with water. Steam would be expected to heat protein bodies from the outside surface toward the center. Formation of an insoluble, denatured layer of protein on the surface would likely retard penetration of moisture into the interior of the protein body. At the same time, the close proximity of proteins contained within the protein bodies would be expected to lend a measure of stability to the remaining undenatured protein. Such an explanation was supported also by the finding that extensive water extraction of steam-treated flours, under the conditions used for the NSI analysis, failed to remove all of the undenatured protein. Since alkali was used successfully to extract all of the undenatured protein, such insoluble surface material would not be expected to contain significant numbers of newly formed covalent bonds. Rather, noncovalent associations probably play a role here as well as in the changes noted (Figs. 4 and 5) for the Kunitz inhibitor.

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