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Effect of ionic strength and pH on the thermal and rheological properties of soy protein–amylopectin blend☆

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Abstract

The effects of amylopectin (AP), moisture content, pH, and buffer molarity on the thermal properties of milled defatted soy flour (MDF) and soy protein isolate (SPI) were studied by differential scanning calorimetry (DSC), and the rheological properties of effect of AP on MDF were also investigated. Native soy protein, which is found in the MDF sample, showed higher stability at alkaline pH, as indicated by higher onset and peak temperature when compared with SPI. The effect of manufacturing isolation process on soy proteins is apparent in the differences between MDF and SPI thermal properties and SDS–PAGE profiles. The onset temperature and ΔH values of the 7S showed significant difference between MDF and SPI at 20% moisture content, 0.3 M and pH 4. The 11S of the MDF and SPI showed that the peak temperature and ΔH to be significantly different at the same buffer amount and pH mentioned earlier. The 30% moisture content generated significant differences between the MDF and SPI under all experimental conditions, i.e. pH and molarity. The increase in the moisture content to 40% produced similar changes as at 30%. The 11S of the SPI sample displayed ΔH values higher than MDF, which may indicate aggregation as a result of the manufacturing process. The amylopectin suspension had strong viscoelastic solid properties and the properties were stable during heating or cooling between 25 and 55 °C. MDF also displayed viscoelastic solid properties, but not as strong as those of AP. The viscoelastic properties of MDF suspensions were not stable and were damaged during heating and cooling processes. Blending MDF and AP exhibited similar properties as MDF alone, but they were reversible during the heating/cooling process, indicating that the networks were not damaged. The strong gel properties of AP in the blend were reduced.
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Keywords: Soy protein isolate (SPI); Milled defatted soy flour (MDF); Soy protein; Amylopectin (AP); DSC; Onset; Peak; ΔH; Viscoelastic properties; Rheology

1. Introduction

The interaction of proteins with water is known to have a major effect on its physicochemical properties. Other factors, such as pH, and ionic strength are also considered to affect protein properties. Functional properties of proteins in food are determined by their structural changes. Protein denaturation or aggregation is often the key to their suitability for certain functionality, e.g. aggregation of proteins in cheese and egg white. Soy proteins are classified according to their sedimentation properties, into four groups, 2S (8%), 7S (35%), 11S (52%), and 15S (5%). Soy protein is known to be heat-stable due to the extensive disulphide bonds between the subunits. This characteristic limits the use of soy proteins in many applications. The diverse composition of soy proteins makes it difficult to set a single temperature where all protein subunits denature or aggregate at the same time (Kinsella, 1979). Srinivasan and Kinsella (1982) reported that the presence of conglycinin prevented glycinin aggregation and formed a soluble complex. The 7S and 11S globulins are stable at ionic strength of 0.5 M and pH 7.6, and associate at 0.1 M ionic strength and pH 7.6 (Hermansson, 1978). Koshiyama (1972) studied the effect of salt on soy protein unfolding and association or aggregation as a result

☆ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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of heat. He concluded that salt might reduce dissociation but did not prevent it. Protein aggregation occurs on heating and is enhanced by salt. Prolonged heating of 11S protein caused aggregation and precipitation of aggregates (Wolf & Tamura, 1969).

Increase of water content allows proteins to hydrate and as a result denature sooner than with less moisture (Hagerdale & Martens, 1976). Fukushima (1969) suggested that soy protein tertiary structure needs to be destroyed to allow its digestion. The presence of a hydrophobic region in the core of soy protein globular structure hardens tertiary structure destruction. The native structure of soy proteins could be destroyed by heating or by pepsin at low pH and by cleaving the disulfide bonds (Boonviscut & Whitaker, 1976).

Soy proteins, in different forms were tested for their solubilities in different pH and ionic strengths (Shen, 1976). This work showed that soy protein solubility could not be used as a measure of denaturation, since soy proteins treated at pH 12 showed an increase in solubility while these proteins were extremely denatured. Denatured proteins at most pH values have more solubility than their native forms.

Utsumi and Kinsella (1985) reported that 11S and 7S, of soybean, produced gels with different formation mechanisms and forces involved in the gel formation. The stabilizing forces of the gels were suggested to be hydrogen bonding, hydrophobic interaction, ionic, and disulphide bonds. The effects of salts, reducing agents, and water-soluble solvent were found to be involved in the gel formation process.

Catsimpoolas and Mayer (1970) showed that soybean protein solution with 8% concentration could form a progel when heated to optimum temperature and a gel when cooled. This process was reported to be reversible. They also reported that pH, ionic strength, and temperature had direct effects on the progel and the gel. Heating the protein solution in excess, extreme acidic, and alkaline pH resulted in no gel formation.

The hardness values or the gel strengths of different globular proteins bear a linear relation to the size and shape of the polypeptides in the gel network (Chen-Hsin & Srinivasan, 1990). Sessa, Nelson, and Snyder (1998) demonstrated that type and concentration of salts affected the nitrogen solubility index and heat-stability of soy protein. The same authors reported that reducing salts, such as sodium sulphite, effectively heat-stabilized 7S and 11S of soybean protein (Sessa & Nelson, 1994).

Changes due to the isolation process may alter soy protein functional properties, such as foaming, which may limit their uses. Conversely, an isolation process that brings about small changes to the protein structure may also benefit soybean utilization.

The objective of this research is to examine possible thermal and conformational differences between soy protein isolate (SPI) and milled defatted soyflour (MDF), using differential scanning calorimetry (DSC), SDS-PAGE and rheology as means of comparison.

2. Materials and methods

2.1. Materials

Protein Technology International, St. Louis, MO, supplied EDI Pro A, a food grade soy protein isolate with 87.3% protein content (N×6.25) and 5.3% moisture (this will be called "SPI sample"). A commercial sample of soy flour, purchased from a local store and produced by Hodgson Mill, Effingham, IL, was used for comparison (this will be called MDF). The soy flour sample (4% moisture) was sieved through a 230-mesh screen to enrich protein content. The sieved soy flour was batch-defatted four times with hexane (1:5 w/v flour:hexane ratio) at room temperature and sieved through a 230-mesh screen and centrifuged (3000 *g* for 20 min) after each hexane extraction. After each centrifugation step, the top layer was scraped off with a spatula before the second hexane extraction. The protein content of the MDF sample was 67% (N×6.25). AP was isolated from common cornstarch using the method of Montgomery and Senti (1958). Starch slurry (20 g/l of water) was added to water at 98 °C while stirring for 11–15 min. The pH of the solution was adjusted from 6.0 to 6.3. The solution was stirred for 5 min and cooled to room temperature in an ice bath. The cooled solution was centrifuged at 2000 *g*. Amylose was in the supernatant, and AP formed a gel at the bottom of the centrifuge tube. The AP gel was re-dispersed twice in water at 98 °C for 11 min and centrifuged at 2000 *g*. AP was recovered from the gel-like precipitate and blended for 4 s with 300 ml methanol, three times. At the final step the AP material was recovered by filtering with suction and air-dried for 1 day. The air-dried material was passed through a 45-mesh sieve and dried under vacuum oven at 40 °C overnight. Previous studies, using the same procedure, have shown that some amylose is still present in fractionated AP (L. Grant, unpublished data).

2.2. Methods

2.2.1. Differential scanning calorimetry

Five concentrations of SPI or MDF and amylopectin (AP) samples at 50%/50% ratio were prepared. Sample weight was corrected for protein content before calculations. Protein (1 g) and AP (1 g) were mixed well with a spatula in a test tube for 3 min. The same protein–AP blends were mixed with phosphate buffer to final moisture contents of 20, 30, or 40%. Phosphate buffers with pH 4, 7, 9 and 0.3, 0.5, 1.0 M concentration, were used. The level of AP added, in the blend, did not produce any

DSC transition peak under the experimental conditions, including 0.2 μ W/s sensitivity. The DSC 2920 (TA Instrument, New Castl, DE) conditions were set at 5 °C/min from ambient to 190 °C for both SPI and MDF blends with AP. The DSC was calibrated against an indium standard. During each run, nitrogen flow rate was 24 (cm^3/min). Samples were hermetically double-sealed in coated aluminium pans and calculations were made on corrected soy protein content in the sample. A 7–8 mg ±0.1 mg blend was added to the pan and the correct amount of buffer was added to reach the targetted moisture content and the samples were left to equilibrate for 6 h before DSC testing.

2.2.2. SDS–PAGE

Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) was used to examine possible aggregate formation during soy protein manufacturing processes. SDS–PAGE was performed according to Laemmli (1970), as described by Khan, Tammiga, and Lukow (1989) with 11.8% acrylamide and 0.1% bis-acrylamide for the separating gel. The stacking gel was prepared with 4.5% acrylamide and 0.1% bis-acrylamide. Samples were analyzed as native or reduced with dithiothreitol (DTT) to test whether proteins formed aggregates involving disulphide bonds.

2.2.3. Rheological measurements

AP, MDF, and the mixture of AP and MDF were suspended in a 0.3 M potassium phosphate, pH 7.0 (25 °C) via extensive mixing (Xu, Bietz, Felker, Carrier, & Wirtz, 2001). AP, MDF, and a mixture were well dispersed and monitored by optical microscope. The suspensions did not display sedimentation during a 2-week period after sample preparation. Measurements were conducted on freshly made samples. Duplicate suspension samples were made for each concentration.

Rheological properties of AP, MDF, and the mixture of AP and MDF suspensions were measured with a Rheometrics ARES strain-controlled fluids rheometer using a 50-mm diameter cone-plate or parallel plate geometry (Xu et al., 2001). The angle of the cone was 0.04 radians. The edge of the plates was sealed with mineral oil to prevent sample moisture evaporation. The temperature was controlled using a water circulation system. The heating and cooling procedures were set up with 0.5 °C/min rate. The rheological measurements were conducted after allowing the samples to stabilize for 30 min after heating and cooling procedures. Prior to dynamic rheological parameter measurements, a strain/sweep experiment was conducted to ensure that the experiments were conducted in the linear viscoelastic range. Linear viscoelasticity indicates that the measured parameters are independent of applied shear strains. Small-amplitude oscillatory shear experiments were conducted over a frequency (ω) range of 0.1–100 rad/s, yielding the shear storage G′ and loss G″ moduli. The storage modulus represents the non-dissipative component of mechanical properties and is characteristic of elasticity. The loss modulus represents the dissipative component of the mechanical properties and is characteristic of viscous flow. The phase shift (δ) is defined by $\delta = \tan^{-1}(G''/G')$, and indicates whether a material is solid ($\delta = 0$), or liquid ($\delta = 90$), or something in between.

Each measurement was repeated at least twice with different samples. The relative errors were all within the range of ±12%.

2.2.4. Statistical analysis

A completely randomized design with three replications was applied using SAS (1992). Each replication has been adjusted for a control value at the specific level of three experimental variables: percentage of buffer, pH, and molarity. A Levene's homogeneity of variance test was conducted to determine whether any data transformations were necessary for dependent variables of: onset and peak temperatures or ΔH of both DSC transitions of each protein. No transformations were needed. A one-way ANOVA was performed comparing the MDF and SPI blends with AP at each of the levels of experimental variable conditions listed earlier. If a significant F-test result was observed from the ANOVA table, the two treatments were significantly different at the P-value level of the ANOVA.

3. Results and discussion

The conditions of these experiments were selected to cover most of the possible use conditions for soy proteins, i.e. moisture, pH, buffer molarity. The results of some of these conditions on soy proteins have been reported in the literature, but this work was designed to combine and compare the effects of these conditions on both native and manufactured soy protein isolates. Since most of the uses of soy protein will include a heating process in the presence of other ingredients, it is important to study the thermal properties of these proteins. DSC thermograms of the two proteins, blended with 50% AP, generated three measurable parameters, denaturation enthalpy (ΔH), onset, and peak temperatures. Consistent with the data reported by Sessa (1992), soy proteins produced a thermogram with two endothermic peaks. The findings of this work showed that the conditions specified here caused shifting of the thermal properties of both proteins.

Figs. 1 and 2 illustrate the effects of moisture content, pH, and molarity on the thermal properties of MDF and SPI. Higher moisture content decreased the onset, peak temperature, and ΔH of MDF at pH 7 and 9, where ΔH values showed an increase as the moisture

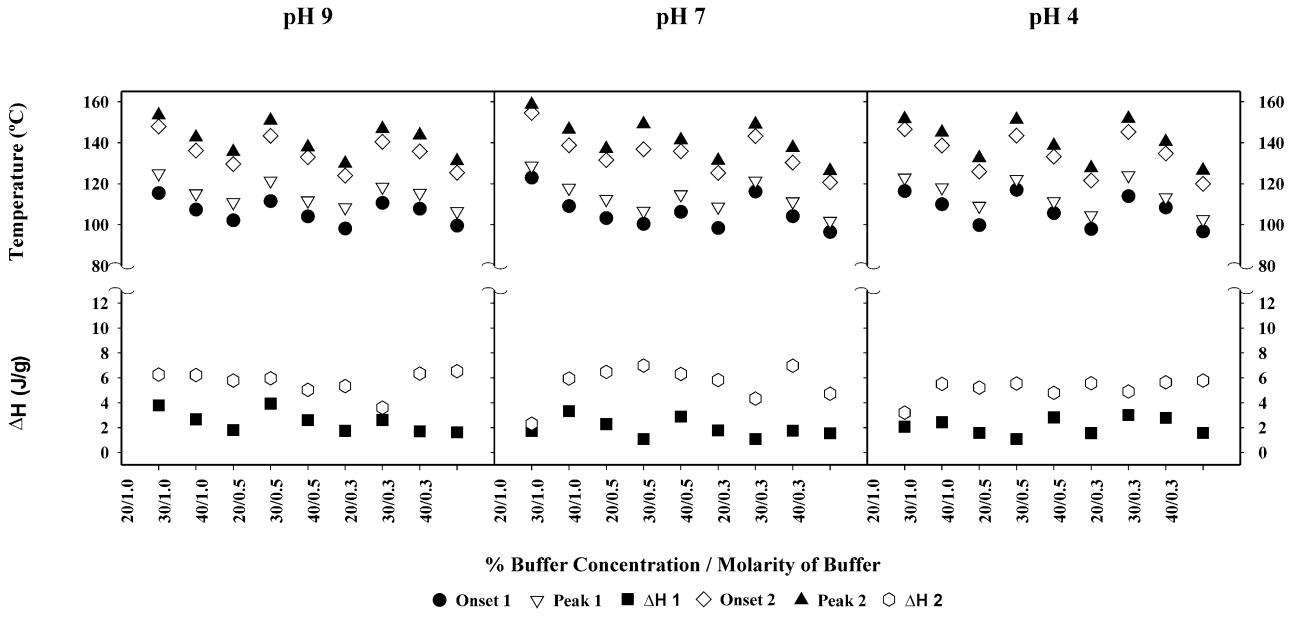

Fig. 1. Thermal properties (onset, peak, and ΔH) of MDF with 20, 30, and 40% moisture contents; pH 4, 7, and 9; 0.3, 0.5, and 1.0 M phosphate buffer.

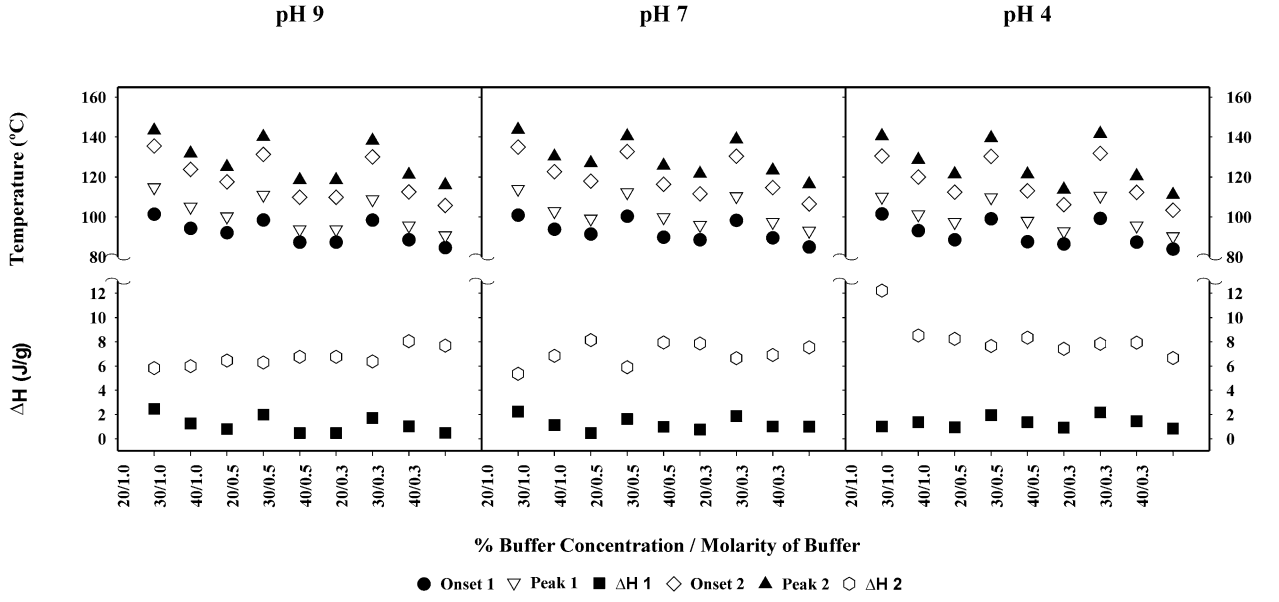

Fig. 2. Thermal properties (onset, peak, and ΔH) of SPI with 20, 30, and 40% moisture content; pH 4, 7, and 9; 0.3, 0.5, and 1.0 M phosphate buffer.

increased. This trend is in agreement with Sessa's (1992) report, but the values reported here for the temperature were higher for both proteins and the ΔH values were lower for 11S. The reason for this difference could be the difference in the soy flour source, which in turn affects protein hydration. A higher buffer molarity increased the stability of the protein as was reflected by higher onset and peak temperatures of MDF as the molarity rose. Native soy protein was more stable in alkaline pH. This was apparent from the gradual increase of the denaturation temperature and the ΔH at pH 9 when compared with pH 4 and 7 (Figs. 1 and 2). The onset and peak temperatures of SPI were lower than MDF. This indicates structural changes as a result of the isolation step during manufacturing. The trend of higher stability of these proteins at lower moisture and higher molarity is valid for SPI as well, but the ΔH values of 11S (ΔH_2) indicate otherwise. For these, lower molarity resulted in higher ΔH at pH 7 and 9 (Fig. 2). Shen (1976) reported that SPI solubility increased with rising ionic strength at pH 4.7 and decreased slightly at pH 6.8 or decreased sharply at pH 2. He also reported

that loss of solubility (salting out) of SPI was an indication of denaturation or aggregation, which may explain why SPI samples used here have higher ΔH_2. Privalov and Khechinashvill (1974) reported that there was a positive correlation between higher denaturation temperature and higher ΔH of globular proteins. The data for MDF reported here are in agreement with this report, but SPI showed bigger differences between ΔH values than between temperatures, probably due to the manufacturing isolation process of SPI.

The differences of temperatures and ΔH between SPI and MDF samples (Figs. 1 and 2), suggest structural differences between the two proteins due to the manufacturing process, as reported in a previous paper (Mohamed, 2001). These structural differences were obvious on SDS–PAGE (Fig. 3), where aggregates were present in the manufactured sample.

An example of DSC thermograms of soy protein and AP blends with different treatments is shown in Fig. 4. The effect of AP with soy protein at various moisture contents, adjusted by a phosphate buffer to changed levels of pH and molarity, is shown in Fig. 4. The DSC data generated by the blends were statistically analysed and compared. The analysis showed that, at 20% moisture, 0.3 M, and pH 4, the 7S onset temperature and ΔH_1 were significantly different while 11S showed peak temperature and ΔH_2 to be significantly different at $\alpha = 0.05$ (Fig. 5). As the moisture content was kept

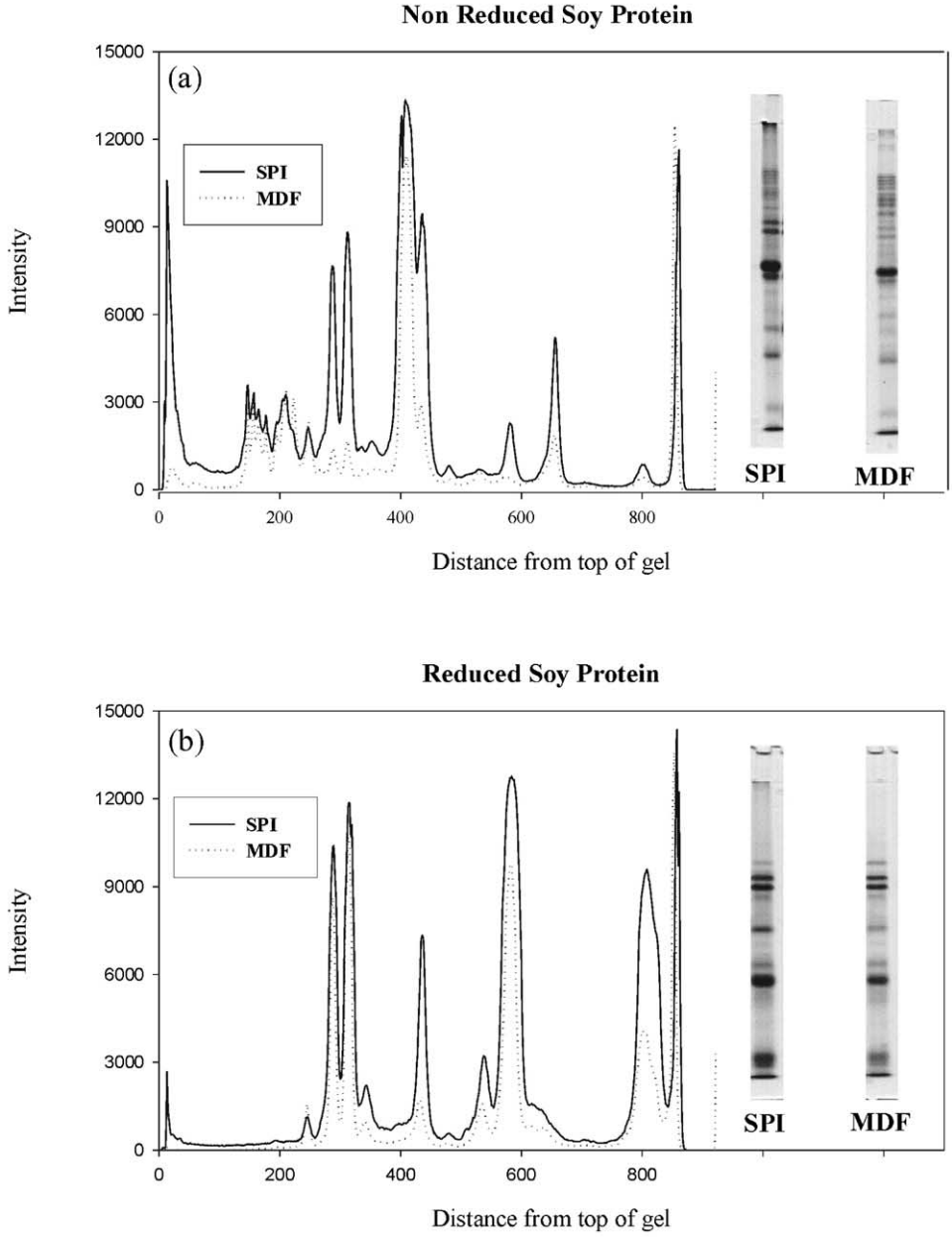

Fig. 3. SDS–PAGE profiles of SPI and MDF soy protein. (a) Reduced (b) non-reduced.

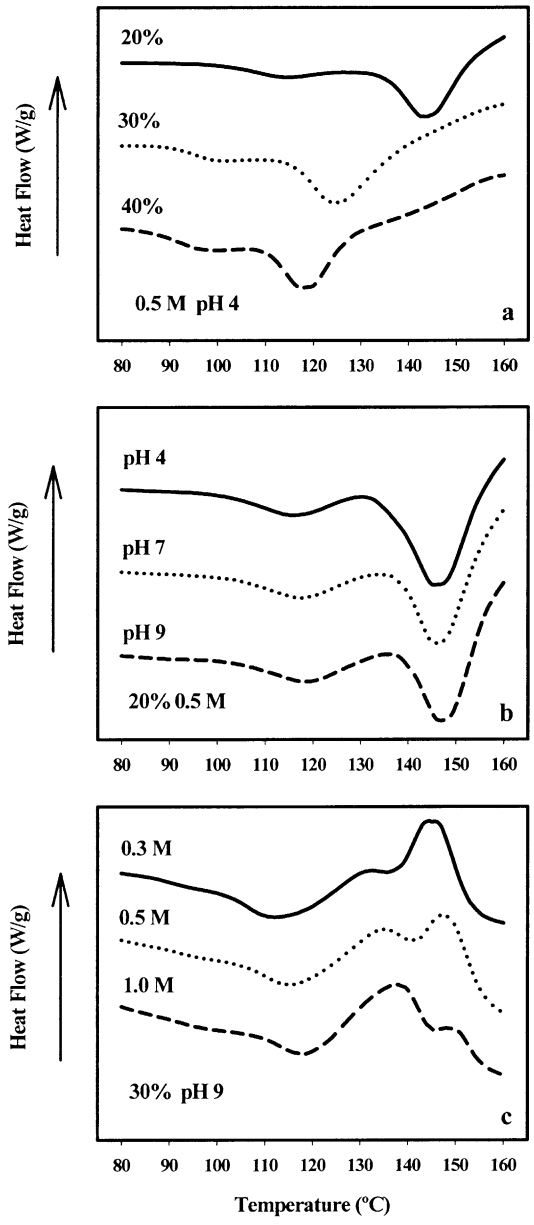

Fig. 4. DSC thermograms of MDF/AP (50%/50%) and SPI/AP (50%/50%) Blends (a) SPI blend with 20, 30, or 40% moisture content, pH 4, and 0.5 M phosphate buffer; (b) SPI blend with pH 4, 7, 9 or 20% moisture content and 0.5 M phosphate buffer; (c) MDF blend with 0.3, 0.5, or 1.0 M phosphate buffer, 30% moisture content, and pH 9.

constant and the pH and molarity were changed, no noticeable difference between the two proteins was revealed under these conditions due to the low moisture content where the protein was more stable and less reactive as a result of the low moisture. Sessa (1992) and Kitabatake, Tahara, and Doi (1990) reported that 7S DSC peak disappeared at low moisture content indicating soy protein stability at low moisture content. This stability was not observed when a purified 7S was tested, as reported by Sessa (1992), but soy flour testing was in agreement with Kitabatake et al. (1990) findings.

With moisture content increase, more significant differences were noticed between MDF and SPI at similar pH and molarities. The two proteins showed significant differences between temperatures and ΔH when tested at 30% moisture, at all three levels of molarity and pH. In some situations we noticed significant difference between temperatures but not between ΔH values, i.e. at 30% moisture, 1.0 molarity, and pH 9. These differences indicate variation in the shape of the DSC transition peak, which in turn indicate different denaturation mechanisms of soy protein under these conditions, i.e. broadening or steepness of the DSC peak. At 40% moisture content, the two proteins displayed the same trend as the 30% moisture content. This is in agreement with Hagerdale and Martens (1976) report of the diminishing effect of moisture contents higher than 30% on protein denaturation.

At pH 4, SPI showed overall lower denaturation temperatures than at pH 7 and was also lower than MDF at all three pH values (Figs. 5–7). This might indicate structural changes as a result of manufacturing isolation procedure. The 11S of the SPI samples displayed ΔH_2 values higher than those of MDF, possibly due to aggregation during manufacturing. The low solubility of SPI at low pH and 0.6 M was reported by Shen (1976), which indicated aggregation of the 7S and 11S. The variability between MDF and SPI was well established by Nash and Wolf (1967). When 11S protein generates a DSC transition peak below 100 °C this temperature must be the result of quaternary structure dissociation and not subunit denaturation transitions, (German, Damondaran, & Kinsella, 1982). Figs. 5–7 show all 11S values higher than 100 °C but some SPI values were lower than MDF, which indicates partial quaternary structure dissociation, especially with the increase in the moisture content. The values reported by German et al. (1982) were for 11S separated from 7S, unlike our samples.

Suspensions of AP, MDF, and a 50:50 blend of both showed varying rheological behaviours. The effect of rheology on the oscillatory storage (G') and loss (G'') moduli of 10% AP suspension is illustrated in Fig. 8. This suspension exhibited strong viscoelastic solid properties at 25 °C. The storage moduli had a plateau at 500 Pa (Fig. 8A). The phase shifts were 3.9–9.7. The properties of the AP suspension were independent of temperature between 25 and 55 °C regardless of whether the sample was subjected to heating or cooling processes (Fig. 8B and C). This implied that the structure of amylopectin was stable and could not be altered over this temperature range. MDF suspensions showed weaker viscoelastic properties than those of AP (Fig. 9). Ten per cent MDF suspension storage moduli displayed a plateau around 75 Pa (Fig. 9A) and the phase shifts were 11.4–16.0. MDF suspensions also displayed viscoelastic solid-like properties. After heating from 25 to

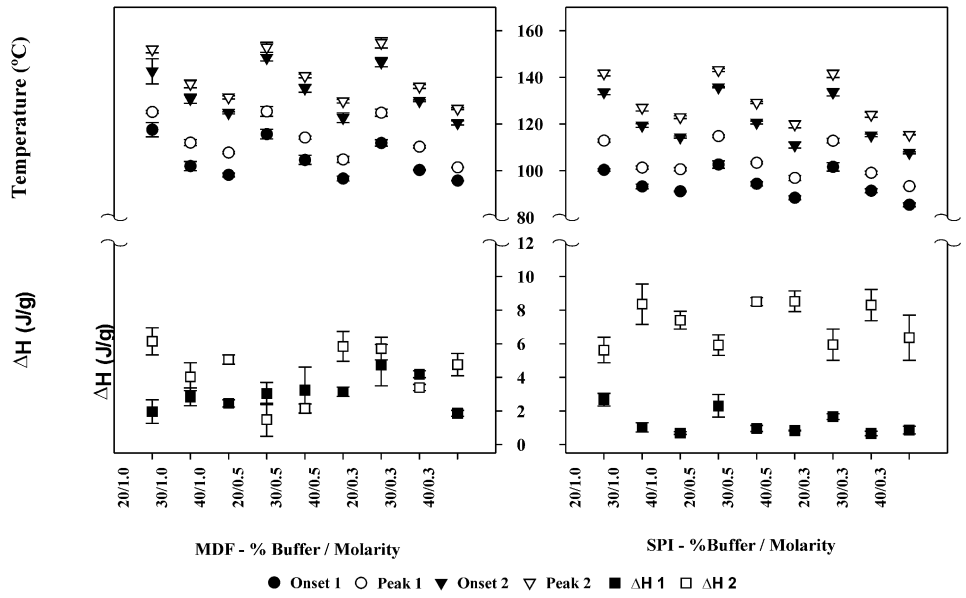

Fig. 5. Thermal properties (onset, peak, and ΔH) of MDF and SPI blends with amylopectin and 20, 30, or 40% moisture content; pH 4; 0.3, 0.5, or 1.0 M phosphate buffer.

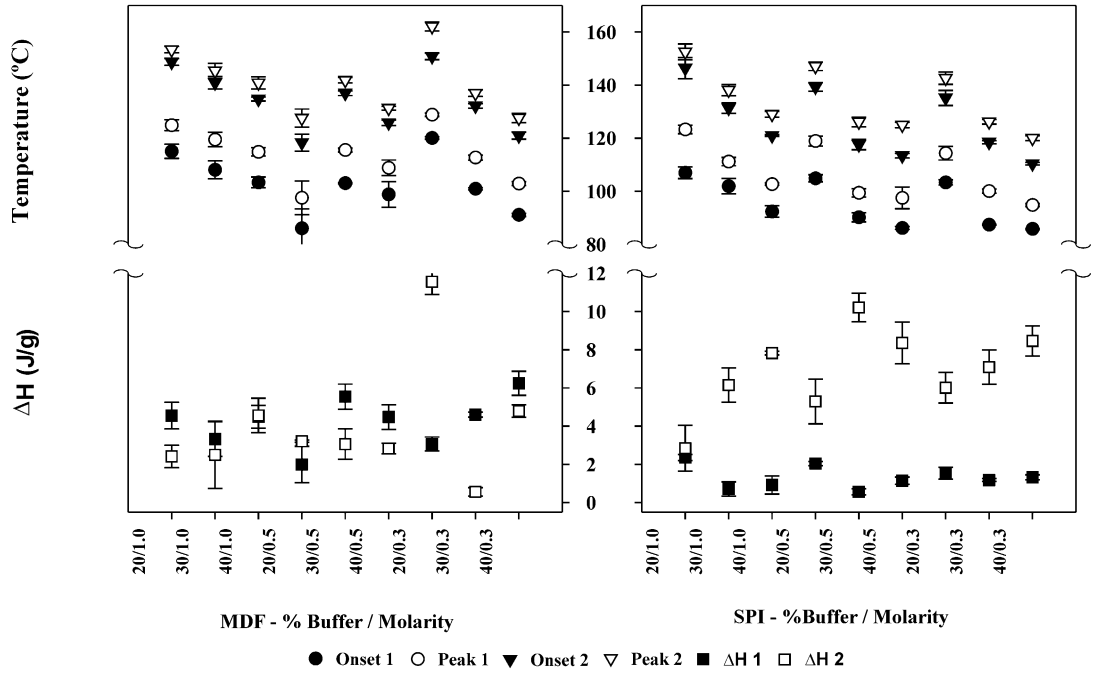

Fig. 6. Thermal properties (onset, peak, and ΔH) of MDF and SPI blends with amylopectin and 20, 30, or 40% moisture content; pH 7; 0.3, 0.5, or 1.0 M phosphate buffer.

55 °C, both G' and G'' of the MDF suspension increased by less than one order of magnitude (Fig. 9B), but the phase shifts were not changed. This indicated that the soybean protein network experienced molecular rearrangement and possibly formed a progel (an early step of gel formation), as reported by Catsimpoolas and Meyer (1970). Upon cooling from 55 °C down to 25 °C, both G' and G'' dropped below the moduli values obtained before the heat/cooling process (Fig. 9C). The G' of plateau decreased from 75 Pa before heating/cooling to 7 Pa. The phase shifts became 12.2–21.5. These changes imply that the heating and cooling processes damaged the structure of the soybean protein network to some extent. This was not consistent with the report by Catsimpoolas and Meyer (1970) who showed that cooling of the soybean protein progel produced a gel, as measured by the increase in the viscosity using a Brookfield viscometer, which is a reversible

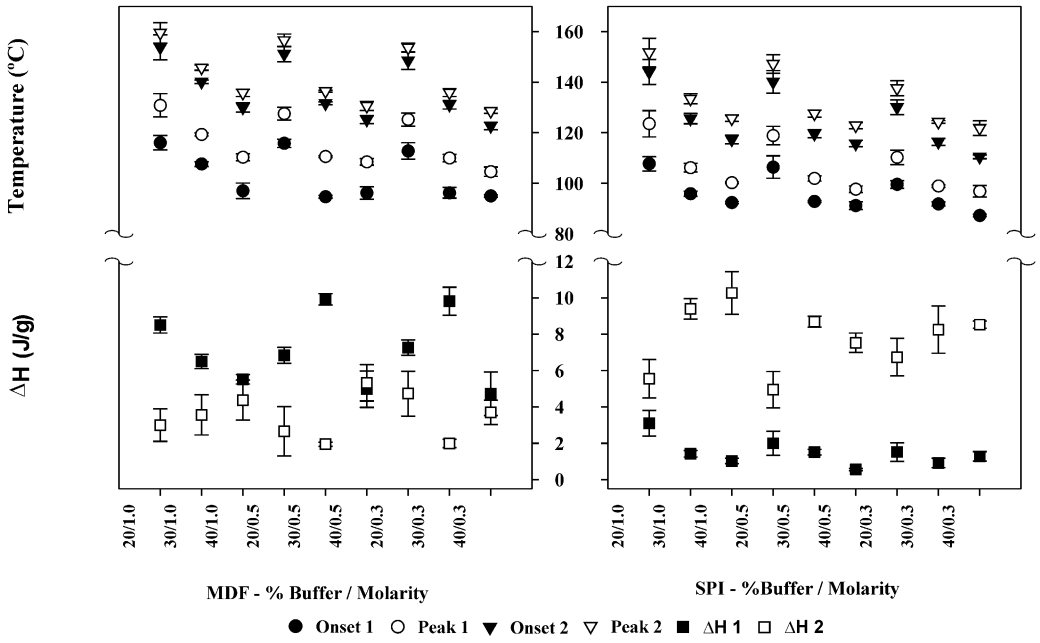

Fig. 7. Thermal properties (onset, peak, and ΔH) of MDF and SPI blends with amylopectin and 20, 30, or 40% moisture content; pH 9; 0.3, 0.5, or 1.0 M phosphate buffer.

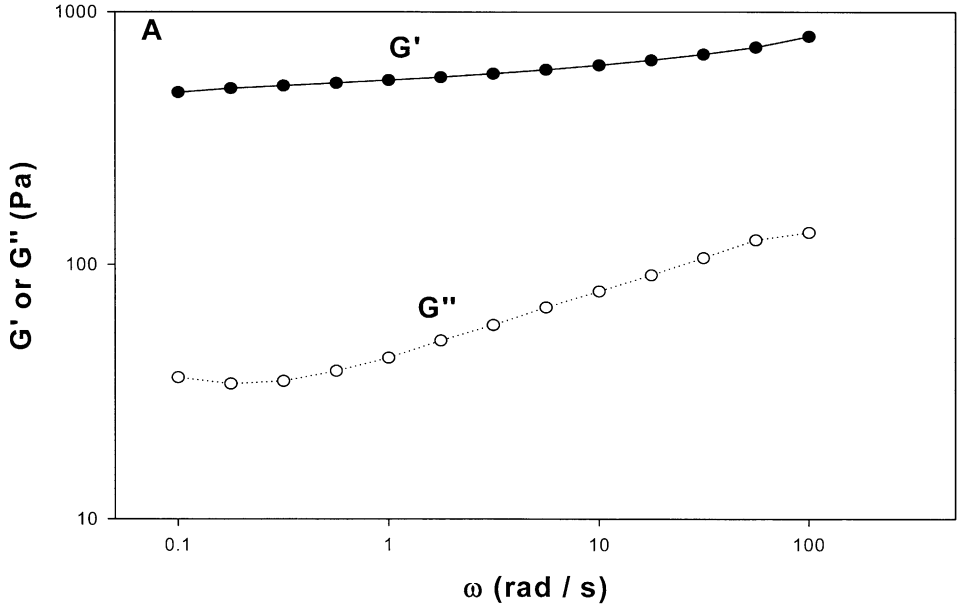

Fig. 8. Storage (G') or loss (G'') moduli for 10% amylopectin suspensions; filled symbol G', opened symbol G''. A: 10% Amylopectin suspension at 25 °C. B: 10% Amylopectin suspension at 55 °C after heating from 25 to 55 °C. C: 10% Amylopectin suspension at 25 °C after cooling from 55 °C back to 25 °C.

phenomenon, i.e. heating of the gel results in progel formation. The difference between the two reports could be due to the difference between the protein systems used; they used purified soybean globulins instead of MDF. The suspensions of the 10% MDF and 10% AP blend exhibited interesting properties. The G'' and G'' of the MDF–AP blend suspensions were the same as the MDF suspensions alone (Fig. 10A). The AP's strong gel properties totally dominated. Heating the blend from 25 to 55 °C caused similar network re-arrangements of the MDF and, both G' and G'' of the blend increased 10 times (Fig. 10B) relative to heating at 25 °C (Fig 10A). After cooling from 55 to 25 °C, both G' and G'' were back to the original values before the heating–cooling process (Fig. 10C). The MDF and AP blend suspensions were stable during the heating–cooling process between 25 and 55 °C; changes in the network were reversible over this temperature range. Apparently,

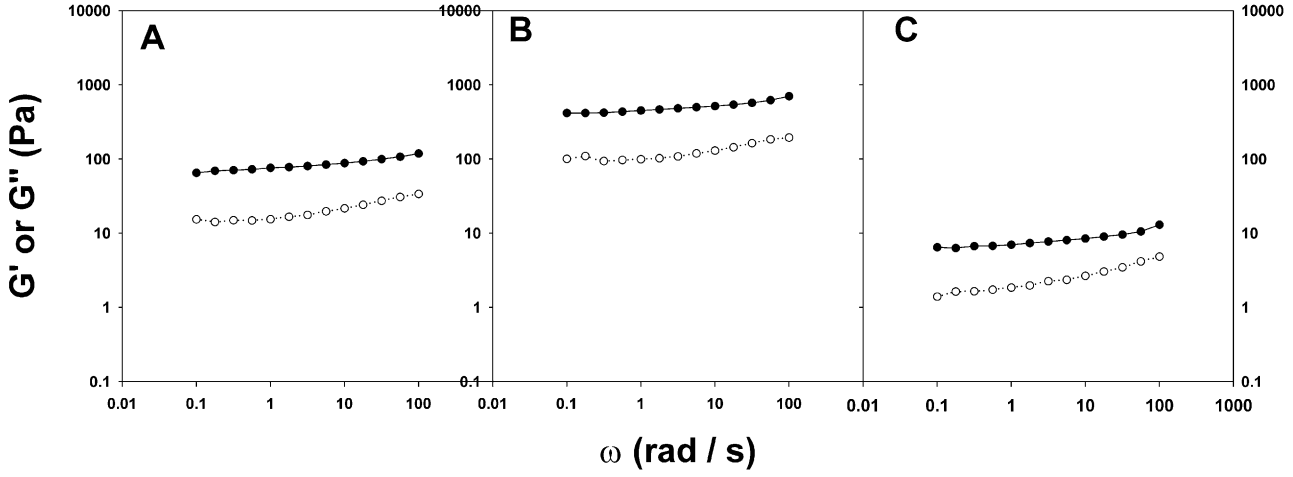

Fig. 9. Storage (G') or loss (G'') moduli for 10% defatted soybean protein suspensions; filled symbol G', opened symbol G''. A: 10% Defatted soybean protein suspension at 25 °C. B: 10% Defatted soybean protein suspension at 55 °C after heating from 25 to 55 °C. C: 10% Defatted soybean protein suspension at 25 °C after cooling from 55 °C back to 25 °C.

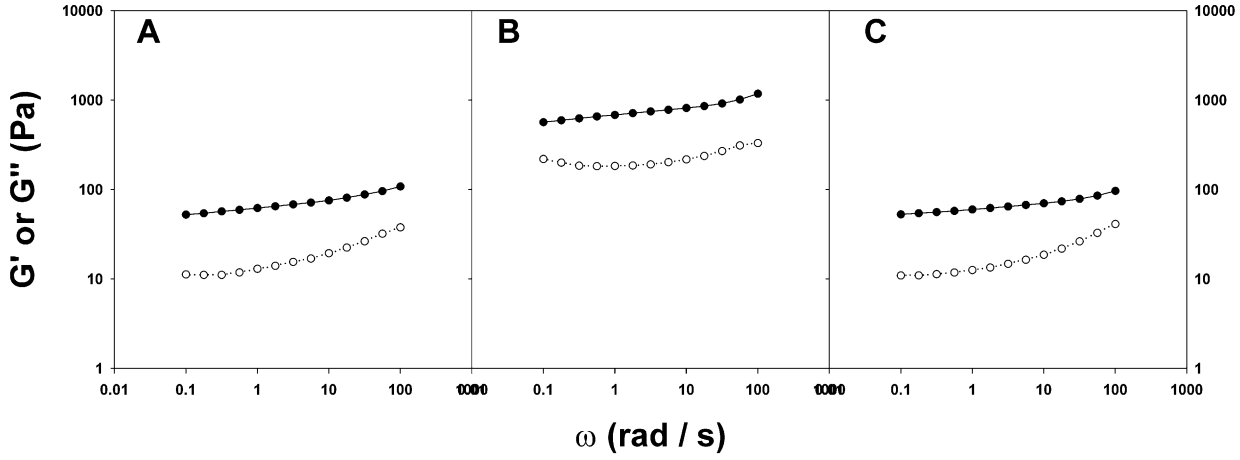

Fig. 10. Storage (G') or loss (G'') moduli for the mixture of 10% amylopectin and 10% defatted soybean protein suspensions; filled symbol G', opened symbol G''. A: The mixture suspension at 25 °C. B: The mixture suspension at 55 °C after heating from 25 to 55 °C. C: the mixture suspension at 25 °C after cooling from 55 °C back to 25 °C.

there are interactions occurring between MDF and AP; MDF network was more stable and the rheological properties of AP were marginalized.

In summary, AP suspension was a strong viscoelastic solid, and its properties were very temperature stable during heating or cooling between 25 and 55 °C. MDF suspension also displayed viscoelastic solid-like properties, but not as strong as those of AP. The viscoelastic properties of MDF suspensions were not stable during the heating and cooling processes. The structure of the MDF network was altered to some extent during the heating/cooling procedure between 25 and 55 °C. However, blending of MDF and AP protected the MDF network. The blend of MDF and AP exhibited similar properties as MDF alone, and the properties were reversible during the heating/cooling process. The strong gel properties of AP in the blend were eliminated. There should be strong interactions between AP and MDF. Further biochemical analysis of the blend is needed to explain the nature of the network.

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References

Boonvisct, S., & Whitaker, J. (1976). Effect of heat, amylase, and disulfide bond cleavage on the in vitro digestability of soy bean proteins. *Journal of Agricultural and Food Chemistry*, *24*(6), 1130–1135.

Catsimpoolas, N., & Mayer, E. W. (1970). Gelation of soybean globulins 1. Protein–protein interactions. *Cereal Chemistry*, *47*, 559–570.

Chen-Hsin, W., & Srinivasan, D. (1990). Thermal gelation of proteins: weight-average molecular weight dependence of gel strength. *Journal of Agricultural and Food Chemistry*, *38*(5), 1157–1164.

Fukushima, D. (1969). Internal structure of 7S and 11S blobulins molecules of soy bean proteins. *Cereal Chemistry*, *45*, 203–224.

German, B., Damondaran, S., & Kinsella, J. E. (1982). Thermal dissociation and association behavior of soy proteins. *Journal of Agricultural and Food Chemstry*, *30*, 807–811.

Hagerdale, B., & Martens, H. (1976). Influence of water content on the stability of myoglobin to heat treatment. *Journal of Food Science*, *41*, 933–937.

Hermansson, A. M. (1978). Physico-chemical aspects of soy proteins structure formation. *Journal of Texture Studies*, *9*, 33–58.

Khan, K., Tammiga, G., & Lukow, O. (1989). The effect of wheat flour on mixing and baking-correlations with protein fractions and high molecular weight glutenin subunit composition by gel electrophoresis. *Cereal Chemistry*, *66*, 391–396.

Kinsella, J. E. (1979). Functional properties of soy proteins. *Journal of American Oil Chemist Society*, *56*, 242–258.

Kitabatake, N., Tahara, M., & Doi, E. (1990). Thermal denaturation of soybean protein at low water content. *Agriculture Biological Chemistry*, *54*, 2205–2212.

Koshiyama, I. (1972). Purification and physico-chemical properties of 11S globulin in soy bean seeds. *International Journal of Peptide & Proteins Research*, *4*, 167–176.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*, 680–686.

Mohamed, A. A. (2001). Effect of corn oil and amylose on the thermal properties of native soy protein and commercial soy protein isolate. *Food Chemistry*, *78*, 291–303.

Montgomery, E. M., & Senti, F. R. (1958). Separation of amylose and amylopectin of starch by an extraction-sedimentation procedure. *Journal of Polymer Science*, *28*, 1–9.

Nash, A. M., & Wolf, W. J. (1967). Solubility and ultracentrifugal studies on soybean globulins. *Cereal Chemistry*, *44*, 183–191.

Privalov, P. L., & Khechinashvilli, N. N. (1974). A thermodynamic approach to the problem of stabilization of globular proteins structure: a calorimetric study. *Journal of Molecular Biology*, *86*, 665–684.

SAS Institute. (1992). *SAS user's guide: statistics*. Cary, NC: The Institute.

Sessa, D. J. (1992). Hydration effects on the thermal stability of protein in cracked soybean and defatted soy flour. *Leb-ensmittel-Wissenschaft und-technologie*, *25*, 365–370.

Sessa, D. J., & Nelson, T. C. (1994). Thermal Stability of Soy Storage proteins in Cracked Beans hydrated with a variety of salts. *Leb-ensmittel-Wissenschaft und-technologie*, *27*, 556–563.

Sessa, D. J., Nelson, T. C., & Snyder, J. M. (1998). Effect of salts on soy proteins defatted with supercritical CO_2 and alcohols. *Journal of American Oil Chemist Society*, *8*(75), 911–916.

Shen, L. J. (1976). Solubility profile, intrinsic viscosity, and opical rotation studies of acid precipitated soy protein and commercial soy isolate. *Journal of Agricultural & Food Chemstry*, *24*(4), 784–789.

Srinivasan, D., & Kinsella, J. (1982). Effect of conglycinin on the thermal aggregation of glycinin. *Journal of Agricultural Chemistry*, *30*, 812–817.

Utsumi, S., & Kinsella, J. (1985). Forces involved in soy protein gelation: effect of various reagents on the formation, hardness and solubility of heat-induced gels made from 7S, and 11S, and soy isolate. *Journal of Food Science*, *50*, 1279–1282.

Wolf, W. J., & Tamura, T. (1969). Heat denaturation of soy bean 11S protein. *Cereal Chemistry*, *46*, 331–344.

Xu, J., Bietz, J. A., Felker, F. C., Carriere, C. J., & Wirtz, D. (2001). Rheological properties of vital wheat gluten suspensions. *Cereal Chemistry*, *78*(2), 181–185.