

Thermal Behavior and Nonfreezing Water of Soybean Protein Components¹

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ABSTRACT

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Thermal denaturation and hydration of two soybean protein components were studied using differential scanning calorimetry (DSC). Results showed that temperature of denaturation (T_d) of both 11S and 7S globulins decreased sharply with an increase in water content. Enthalpy of denaturation (ΔH_d) of 11S increased with increasing water content at first, and then leveled off at high water content. However, ΔH_d of both 7S and 11S components in 7S samples first increased and then decreased at high water content. The preparation method of samples influenced the ΔH_d value significantly but had little effect on the T_d . Nonfreezing water

was determined from the DSC results. It increased in both 11S and 7S as water content increased but was more abundant in 7S, probably because of different compositions and structures. Threshold value of water content for the appearance of freezing water was 0.30–0.32 h (g of water/g of protein, mass ratio) for 11S. The water absorbed by both 11S and 7S during denaturation increased quickly at low water contents and remained almost constant at high water contents. The results were attributed to different structure and conformation of proteins before and after denaturation.

Protein-water interactions play an important role in the determination and maintenance of the three-dimensional structure of proteins, which affects their physicochemical and functional properties and behavior during processing, storage, preparation, and consumption. Many studies on protein-water interactions have been done and have provided significant advances in understanding the involvement of water in protein structure, stability, dynamics, and function (Kinsella et al 1985, Nielsen 1985, Rupley and Careri 1991, Gregory 1995, Roos 1995). Water is essential for the formation of native protein structure (Kauzmann 1951, Edsall and McKenzie 1983) and greatly influences the denaturation of proteins. With increasing water content, more water molecules interact with protein to form a hydration shell, and molecular mobility in the system is improved. The denaturation temperature as well as the thermal stability decreases significantly with an increase of water concentration.

Differential scanning calorimetry (DSC) is a useful method to study thermal behavior of proteins (Wright 1982) and can provide much information, such as the effects of solvent conditions and intermolecular interactions on protein denaturation or the effects of processing conditions on protein functionality. The thermal behavior of soybean proteins also has been studied extensively. Salt suppressed aggregation as well as denaturation of proteins and stabilized the quaternary structure (Hermansson 1978). Addition of calcium also improved the thermal stabilization of soybean proteins (Scilingo and Anon 1996). However, other conditions favoring denaturation, such as high and low pH, had the opposite effect on aggregation (Hermansson 1978, Arntfield and Murray 1981). Studies of the effects of hydrocolloids on soybean proteins with DSC (Oates et al 1987) showed an increase in the apparent water content when soy isolates containing mannuronate alginates were heated. This reaction explained why alginate addition reduced the viscosity of soy proteins during extrusion. DSC results also showed that the specific heat function associated with denaturation of the 7S globulin increased in most of soy and soy + hydrocolloid systems (Oates and Ledward 1991). Interaction of soybean proteins and other additives, including reducing agents such as 2-mercaptoethanol, has been studied. Marshall and Zarins (1989) reported that reduction of intermolecular disulfide bonds between acidic and basic polypeptides did not alter the thermal stability of soybean proteins.

Glass transition of soy protein also has been studied. Morales and Kokini (1997) reported that glass transition temperature of soy protein decreased with increasing water content.

Water is the primary plasticizer used in processing and manufacturing of soybean proteins, and many studies have been done on water content dependency of the thermal properties. Denaturation temperature (T_d) of soybean proteins decreased with increasing water level in the system (Sheard et al 1986; Oates et al 1987; Kitabatake et al 1990; Sessa 1992, 1993), whereas denaturation enthalpy (ΔH_d) displayed some complicated relationships with water level (Oates et al 1987, Kitabatake et al 1990, Sessa 1992). Kitabatake et al (1990) observed no dependency of enthalpy on the water contents of both 11S and 7S proteins. However, other studies showed that ΔH_d for the 11S protein dropped with increasing water content, whereas ΔH_d for 7S protein increased first, and then decreased as water content increased (Oates et al 1987, Sessa 1992). The effect of water content on the inactivation of soybean Kunitz trypsin inhibitor (KTI) also was studied with DSC (Vooijs et al 1989). In these studies, great efforts have been made to understand the relationship between the thermal behavior of soybean protein and the additives. This information is basic not only to fundamental research on the structure-property relationships of proteins but also to food processing and manufacturing. Knowledge of the thermal behavior of soybean proteins during extrusion, toasting, and frying is very helpful for designing processes to achieve products with different appearance, color, flavor, and texture.

However, the effect of sample preparation method on thermal behavior has not been studied, and little information is available about water state in the proteins, such as nonfreezing water (or bound water). The term "bound water" is used widely but is not defined uniformly. It may denote water that does not freeze at a specific temperature, water that is not available as solvent, or water that shows different physical properties. Bound water is really that water in the vicinity of a macromolecule whose properties differ detectably from those of bulk phase water in the same system (Kuntz and Kauzmann 1974, Chou and Morr 1979, Kinsella et al 1985). The determination of nonfreezing water is the most simple and straightforward way to estimate hydration (Kuntz and Kauzmann 1974, Rupley and Caeri 1991). The extent of protein hydration correlates strongly with the content of polar residues and charged residues (Kinsella et al 1985). The study of bound water (i.e., nonfreezing water) can provide important information on the structure and functional properties of proteins (Hermansson 1977; Hansen 1978a,b). It also can be used in establishing proper processing and storage conditions for dehydrated and frozen foods (Roos 1995).

The objectives of this work were to investigate systematically the thermal behavior of two soybean protein components at various hydration levels and large temperature scan ranges, and to determine nonfreezing water fractions as affected by protein denaturation

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and water contents. Both DSC and nuclear magnetic resonance are used widely to measure nonfreezing water (Rupley and Careri 1991). DSC was chosen for this study, and the effect of sample preparation method on thermal behavior also was explored.

MATERIALS AND METHODS

Materials

The soybean protein fractions 11S and 7S were extracted from defatted soybean flour (Cargill, Cedar Rapids, IA) following the procedures described by Sun et al (1999). The 11S and 7S fractions had purity of ≈ 90 and 70% (Sun et al 1999), respectively,

and a moisture content of ≈ 5 wt% as measured by an air-oven method (AACC 2000).

Sample Preparation

Soybean protein samples with different moisture contents were prepared using three methods. Method I: The soybean protein was exposed in a desiccator with 95% moisture atmosphere for various times to obtain samples with moisture contents of 6–32 wt%. Method II: The soybean protein was dissolved first in distilled water to form 20 wt% solution. Then this solution was freeze-dried for various times to obtain moisture contents of 1–76 wt%. Method III: The soybean protein was added to a given volume of distilled

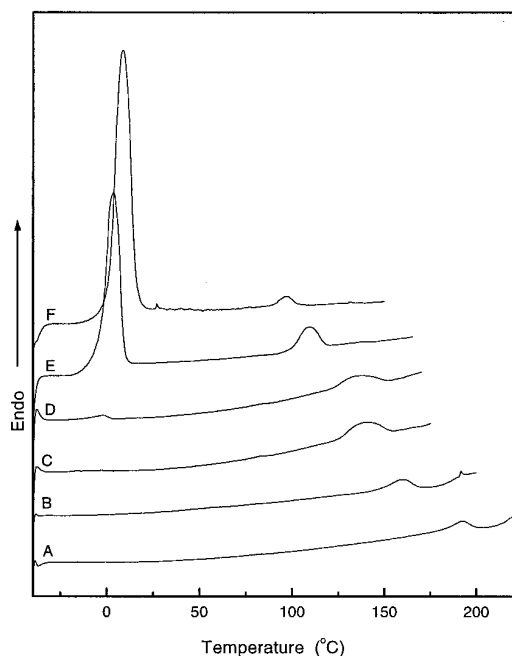


Fig. 1. Differential scanning calorimetry (DSC) thermograms of 11S with water contents (wt%) of 1, 18, 23, 24, 45, and 72 (A–F, respectively). Samples were prepared using methods I and II.

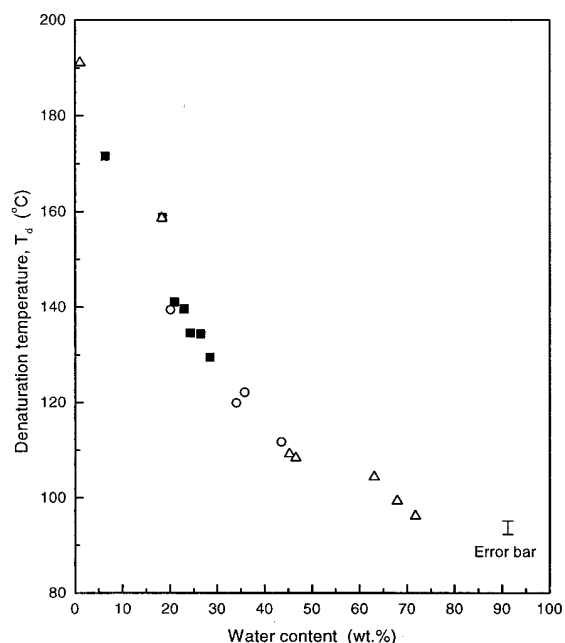


Fig. 2. Relationship of denaturation temperature (T_d) of 11S to water content. Samples were prepared using methods I (■), II (△), and III (○).

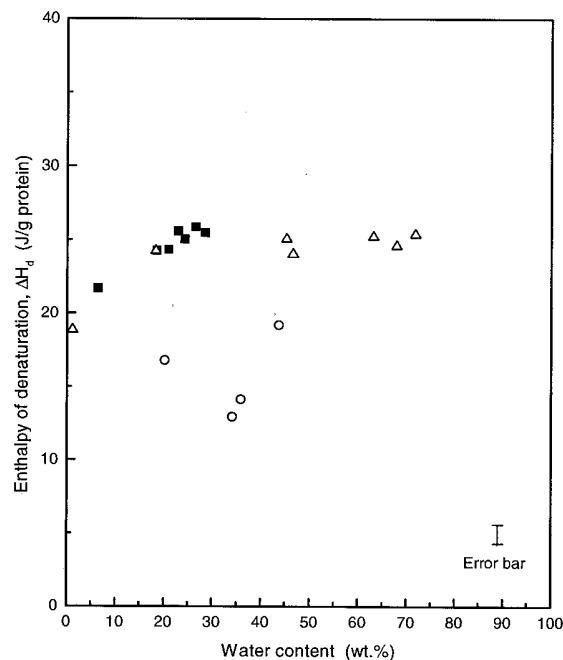


Fig. 3. Relationship of denaturation enthalpy (ΔH_d) of 11S to water content. Samples were prepared using methods I (■), II (△), and III (○).

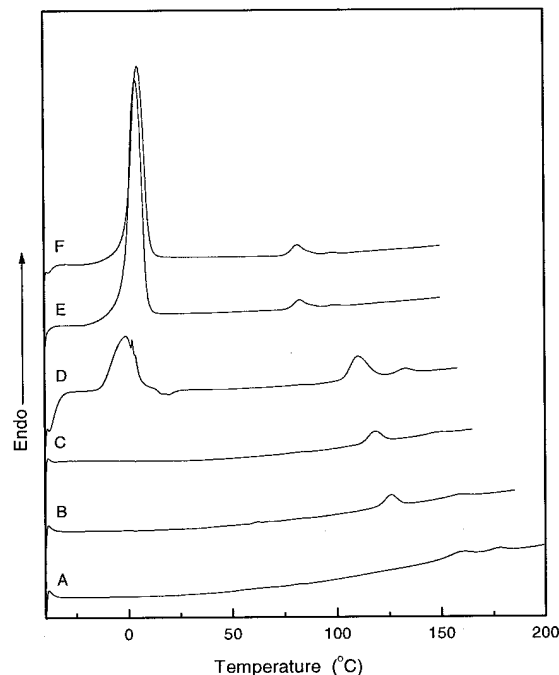


Fig. 4. Differential scanning calorimetry (DSC) thermograms of 7S with water contents (wt%) of 5, 19, 25, 32, 71, and 76 (A–F, respectively). Samples were prepared using methods I and II.

water, and then the mixture was kneaded in a glass mortar with a pestle. The obtained mixtures were sealed and remained at room temperature ($\approx 25^\circ\text{C}$) for at least 48 hr to reach equilibrium before further studies. Moisture contents of samples prepared using this method were 20–44 wt%. All the samples obtained were sealed and stored in a refrigerator until further analysis.

DSC

A differential scanning calorimeter (Pyris-1, Perkin-Elmer, Norwalk, CT) was used for calorimetric measurements. The instrument was calibrated with indium and zinc standards before sample measurements, and all measurements were conducted under a nitrogen atmosphere. All samples first were quenched to -40°C and held at that temperature for 1 min. Then the samples were scanned at $10^\circ\text{C}/\text{min}$ and quenched again to -40°C , held there for 1 min, and reheated at $10^\circ\text{C}/\text{min}$. A few samples were reheated a third time to observe whether any change occurred in nonfreezing water of denatured proteins. The T_d of each sample was taken as the maximum value of the endothermic peak. The melting point temperature (T_m) of frozen water in each sample was taken as the maximum value of the endothermic transition in the vicinity of 0°C . ΔH_d of the protein and the enthalpy of frozen water fusion (ΔH_f) were calculated from the areas encompassed by the baseline and thermogram of protein denaturation and ice fusion, respectively. Because of the overlap of the two peaks, the denaturation enthalpies of the 11S and 7S components in 7S samples were calculated using area-weight of the corresponding peaks. All samples were run in duplicate, and the DSC results were taken as average values of the two scans.

Nonfreezing water was determined using the DSC data following the method described by Ross (1978). The same calorimetric measurements were made for pure water. The melting enthalpy of pure water thus was obtained and used as the reference enthalpy.

RESULTS AND DISCUSSION

Thermal Denaturation

Protein denaturation involves structural or conformational changes from native structure without alteration of the amino acid sequence. These changes can be induced by pH, detergents, urea, and guanidine hydrochloride, as well as by heat treatment. Protein denaturation apparently is a highly cooperative process accompanied by enormous enthalpy changes (Privalov and Khechinashvili 1974, Privalov 1979). All DSC thermograms of 11S during the first scan showed an endothermic peak at $>90^\circ\text{C}$ that corresponded to the denaturation transition of 11S. During the second heating, no peak was observed up to 200°C , indicating that the thermal denaturation of 11S is irreversible.

It has been generally accepted that the compact protein structure unfolds during denaturation. The unfolding is accompanied by breaking and reforming of the intermolecular and intramolecular interactions (Careri et al 1979). Water molecules play an important role in the breaking and reforming of these interactions. These effects will be reflected in the denaturation behaviors of proteins.

The typical DSC thermograms of 11S with various water contents of 1–72 wt% are presented in Fig. 1. Peaks at $\approx 0^\circ\text{C}$ were caused by ice fusion. The endothermic transitions at high temperature were due to thermal denaturation of 11S, which was shifted from high to low temperature as water content increased. The relationship of T_d and ΔH_d to water content of 11S is shown in Figs. 2 and 3, respectively. The T_d decreased sharply with increasing water contents, from 191°C at 1 wt% to 96°C at 72 wt% (Fig. 2), which was in agreement with the results reported by Sheard et al (1986), Kitabatake et al (1990), and Sessa (1993). The ΔH_d of 11S increased as water content increased to 30 wt% and then leveled off at high water content for preparation methods I and II (Fig. 3). The increase in ΔH_d with increase in water content is a common phenomenon for protein denaturation (Gregory 1995). The corre-

lation of T_d and ΔH_d to hydration should reflect contributions from the hydration of both the folded and unfolded states (Rupley and Careri 1991). Reducing the water level would destabilize the unfolded state relative to the native state, because the unfolded species have a more extensive interface with solvent than the folded species. Destabilization of the unfolded state results in a rise in T_d . Presumably, the average conformation in the unfolded state changes as the hydration level is reduced. The unfolded state should be more compact and have more internal bonding at lower hydration levels. Thus, ΔH_d decreases with decreasing water content.

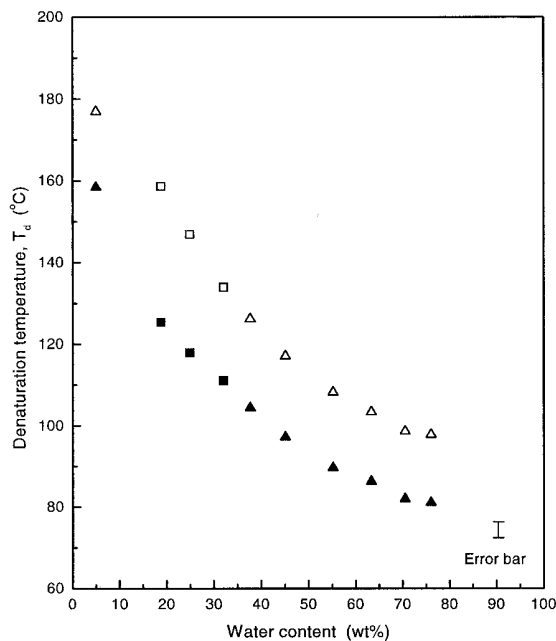


Fig. 5. Relationship of denaturation temperature (T_d) of 7S to water content: 7S component (■ and ▲) and contaminating 11S component (□ and Δ). Samples were prepared using method I (■ and □) and method II (▲ and Δ).

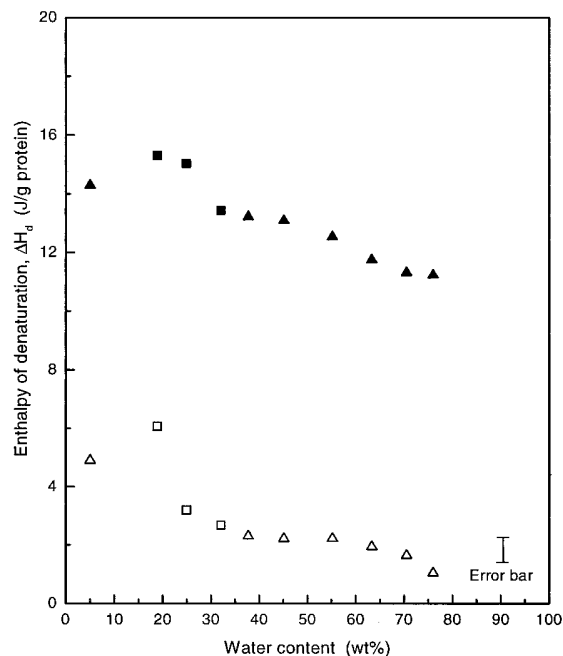


Fig. 6. Relationship of denaturation enthalpy (ΔH_d) of 7S to water content: 7S component (■ and ▲) and contaminating 11S component (□ and Δ). Samples were prepared using method I (■ and □) and method II (▲ and Δ).

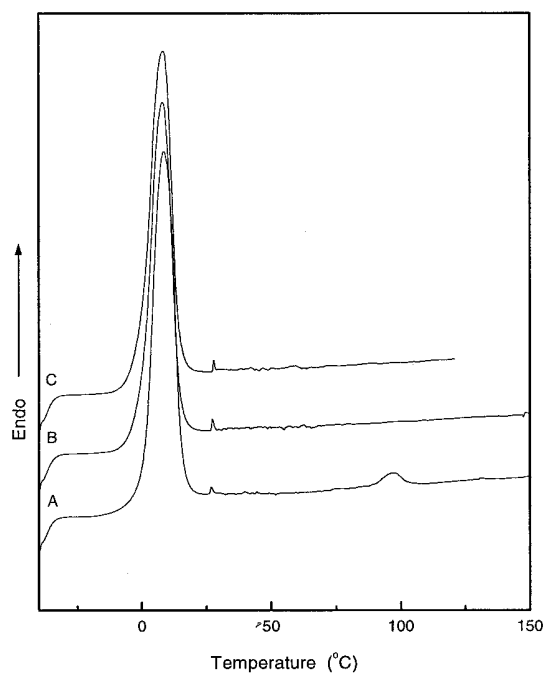


Fig. 7. Differential scanning calorimetry (DSC) thermograms of 11S with 72 wt% water content. First, second, and third scan (A–C, respectively).

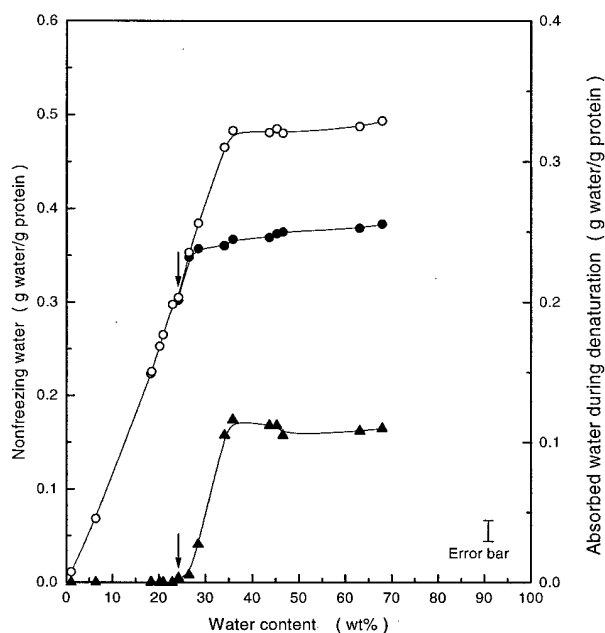


Fig. 8. Variation of nonfreezing water of 11S before (●) and after (○) denaturation and absorbed water during denaturation (▲) with water content. Samples were prepared using methods I, II, and III. Arrows indicate threshold value for appearance of ice-water endothermic transition.

Methods of sample preparation had no obvious influence on the T_d of 11S (Fig. 2). The ΔH_d values for methods I and II did not differ significantly from each other. However, the ΔH_d values for method III were markedly lower (Fig. 3). For example, the ΔH_d was ≈ 19 J/g of protein for the sample with 44 wt% water content prepared using method III, whereas it was ≈ 25 J/g of protein for the sample prepared using method II. The denaturation process is an intramolecular change involving the destruction of internal order. For more complex proteins, like soybean proteins which have a quaternary structure, the process involves dissociation into subunits (Pace 1975). This means that the ΔH_d includes the energy used to break down the quaternary structure and to dissociate the proteins

into subunits. Using viscometry and Fourier transform infrared spectroscopy, Chen et al (1990) found that shear might cause a quaternary or tertiary structure change of soy protein. Using viscometry, UV spectroscopy, and gel chromatography, Pradipasena and Rha (1977) also showed that protein molecules could be unfolded or uncoiled in shearing. Therefore, it is understandable that the samples prepared by kneading (method III) had relatively lower ΔH_d values because the quaternary structure of the 11S proteins presumably was destroyed to some extent during preparation. It should be pointed out that a large difference of ΔH_d between different samples prepared using Method III (Fig. 3) could not be considered as an experimental error because the variation between two DSC duplicates were very small. However, these structural changes during sample preparation did not have a significant influence on T_d . The denaturation stability of the 11S proteins may be due mainly to the stability of the tertiary or secondary structure. Further study on structural changes during sample preparation is needed.

The DSC thermograms of 7S are shown in Fig. 4. Two endothermic transitions occurred at higher temperature. The presence of a minor peak was due to 11S contamination in 7S (Sun et al 1999). The major peak at low temperature represented denaturation of the 7S component, and the minor peak at high temperature represented denaturation of the 11S component. Both peaks shifted to lower temperatures with increasing water content. The T_d of both 11S and 7S components decreased sharply with the increase of water content (Fig. 5). However, the variation of ΔH_d with water content is more interesting (Fig. 6). The ΔH_d of both 11S and 7S components increased with increasing water content up to ≈ 20 wt%, with a subsequent decrease as water content increased further. After that, the ΔH_d was approximately independent of water content. Similar results have been reported by Oates et al (1987) and Sessa (1992).

The variation of ΔH_d of the 11S component in the 7S sample as affected by water content (Fig. 6) differed from that of pure 11S (Fig. 3). This might have been due to an interaction between the 11S and 7S components. However, different water contents and molecular environments also lead to difficulty in comparing these results directly.

Nonfreezing Water

As mentioned before, the endothermic peaks at $\approx 0^\circ\text{C}$ for the 11S samples with high moisture content corresponded to the ice-water fusion transition (Fig. 1). The fusion transition became detectable when moisture contents in the sample were ≥ 24 wt% (Fig. 1). At low moisture content, all water molecules interact strongly with protein molecules and, hence, they cannot freeze under the experimental conditions. However, at high moisture content, some water molecules become relatively free, are not bound tightly by the protein, and have properties similar to the bulk water. These water molecules can freeze under certain conditions. With increasing water content, the fusion peak increased in area (Fig. 1), which was in agreement with previous results (Ross 1978, Muffet and Snyder 1980, Oates et al 1987).

The T_m and ΔH_f of the ice-water transition remained constant after the first heating (Fig. 7). The values were 7.4°C , 189.8 J/g, and 7.4°C , 189.7 J/g for the second and third heating, respectively, whereas the values for the first heating were relatively larger (7.9°C and 197.1 J/g). This indicates that the hydration level in the 11S protein had changed during denaturation and remained the same after denaturation. The changes probably included the protein-water interaction and water redistribution in the system.

For the 11S samples with water contents ≤ 23 wt%, all the water in the samples was nonfreezing (Fig. 8). In the figure, an arrow indicates the threshold value of water content for appearance of ice-water endothermic transition and the minimal water content below which there is no ice formation in the sample. The threshold value of water content was 23–24 wt% (Figs. 1 and 8), which corre-

sponds to 0.30–0.32 h (g of water per g of protein, mass ratio). These values are in agreement with those observed for other globular proteins (0.3–0.35 h) (Kuntz and Kauzmann 1974, Ruegg et al 1975). The nonfreezing water before denaturation and after denaturation increased sharply with increasing water content up to 40 wt%, then remained almost constant (Fig. 8). The sharp increase in nonfreezing water at low water content might have been due to insufficient water in the samples. In addition, the nonfreezing water before denaturation was always lower than that after denaturation (Fig. 8). This is because transition of protein from a compact globular conformation to a random coil results in exposure of previously buried peptide bonds and amino acid side chains that can interact with water (Lewin 1974). Usually, there is an increase of 2–10% of bound water (nonfreezing water) following denaturation (Kinsella et al 1985).

Water absorbed by protein during denaturation was calculated from the difference in nonfreezing water before and after denaturation (Fig. 8). Because nonfreezing water (or bound water) of soy protein increased after denaturation, it seemed that protein absorbed more water after denaturation. The absorbed water during denaturation was zero at low water contents because of insufficient water in the samples. After that, the absorbed water during denaturation increased first with increasing water content and then leveled off at ≈ 40 wt%. With the increase of water content, the conformation of denatured 11S protein became more extensive and exposed more previously buried peptide bonds and side groups. Therefore, the denatured protein absorbed more water molecules. As water content reached ≥ 40 wt%, the conformation of denatured 11S reached its most extensive state, thus water absorption was saturated. Protein aggregation after denaturation actually may decrease water binding by replacing water-protein interactions with protein-protein interactions. This may lead to underestimation of the absorbed water during denaturation. However, the absorbed water during denaturation still can be used to study the structure and conformation changes of proteins or modified proteins.

Nonfreezing water presumably results from competition between the protein surface and growing ice crystals for the water in the interface between the surface and the bulk solvent. Ice crystals would be expected to incorporate interface water over regions of the surface where water does not interact strongly with protein or where the surface can accommodate the ice structure. Thus, the higher the level of nonfreezing water, the stronger the interaction between protein and water.

Similar results were obtained for 7S (Fig. 9). All water in the 7S samples with water contents of ≤ 25 wt% was nonfreezing. This nonfreezing water increased sharply with increasing water content up to 40 wt% and then increased slowly at higher water contents. Compared to 11S, 7S had more nonfreezing water. This could be due to the different compositions and structures of the two proteins. Kuntz (1971) suggested that nonfreezing water is dominated by contributions from water around ionic residues. 7S has higher charge density than 11S (Kinsella et al 1985), and this can result in a greater ability to interact with water molecules. Figures 8 and 9 also show that the values of absorbed water during denaturation for 7S and 11S did not differ significantly.

CONCLUSIONS

Water plays an important role in the thermal behavior of 11S and 7S fractions of soybean proteins. The T_d of both 11S and 7S decreased sharply as water content increased. The ΔH_d first increased for both 11S and 7S with increasing water content but then decreased for 7S and leveled off for 11S at high water contents. The ΔH_d of 11S in 7S samples showed changes in behavior similar to that of 7S. Kneading of protein samples might destroy the quaternary structure resulting in lower ΔH_d compared with that of protein prepared by exposure to water vapor or lyophilizing methods.

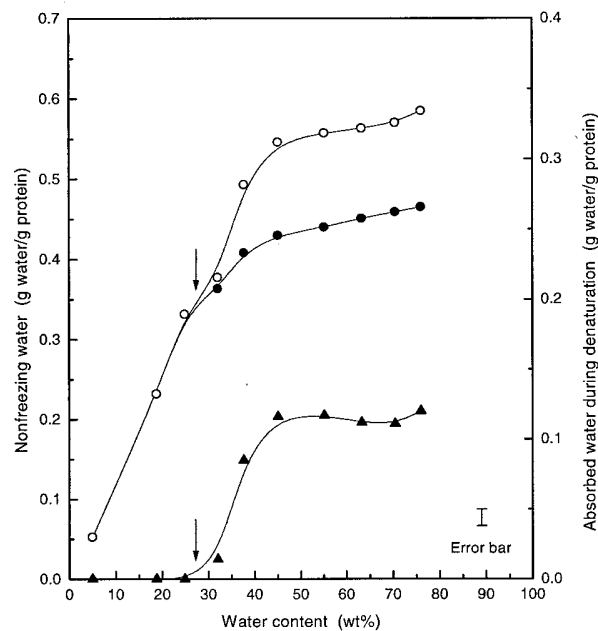


Fig. 9. Variation of nonfreezing water of 7S before (●) and after (○) denaturation and absorbed water during denaturation (▲) with water content. Samples were prepared using methods I and II. Arrows indicate threshold value for appearance of ice-water endothermic transition.

Composition and structure-conformation of proteins had important effects on the nonfreezing water. Nonfreezing water in both 11S and 7S increased with increasing water content but it was more abundant in 7S. The threshold value of water content for the appearance of freezing water was 0.30–0.32 h for 11S. Water absorption occurred during protein denaturation, increased quickly at low water content, and became almost constant at high water content.

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LITERATURE CITED

- American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. Method 44-15A. The Association: St. Paul, MN.
- Arntfield, S. D., and Murray, E. D. 1981. The influence of processing parameters on food protein functionality. I. Differential scanning calorimetry as an indicator of protein denaturation. *Can. Inst. Food Sci. Technol. J.* 14:289-294.
- Careri, G., Giansanti, A., and Gratten, E. 1979. Lysozyme film hydration events: An IR and gravimetric study. *Biopolymers* 18:1187-1203.
- Chen, R.-H., Ker, Y.-C., and Wu, C.-S. 1990. Temperature and shear rate affecting the viscosity and secondary structural changes of soy 11S globulin measured by a cone-plate viscometer and Fourier transform infrared spectroscopy. *Agric. Biol. Chem.* 54:1165-1176.
- Chou, D., and Morr, C. 1979. Protein-water interactions and functional properties. *J. Am. Oil Chem. Soc.* 56:53A-62A.
- Edsall, J. T., and McKenzie, H. A. 1983. Water and proteins. II. The location and dynamics of water in protein systems and its relation to their stability and properties. *Adv. Biophys.* 16:53-183.
- Gregory, R. B. 1995. Protein hydration and glass transition behavior. Pages 191-264 in: *Protein-Solvent Interactions*. R. B. Gregory, ed. Marcel Dekker: New York.
- Hansen, J. R. 1978a. Dehydration and hydration kinetics of soybean proteins. *J. Agric. Food Chem.* 26:297-301.
- Hansen, J. R. 1978b. Hydration of soybean protein. 2. Effect of isolation method and various other parameters on hydration. *J. Agric. Food Chem.* 26:301-304.
- Hermansson, A. M. 1977. Functional properties of proteins for foods—Water vapor sorption. *J. Food Technol.* 12:177-187.
- Hermansson, A. M. 1978. Physicochemical aspects of soy proteins structure formation. *J. Texture Stud.* 9:33-58.

- Kauzmann, W. 1951. Some factors in the interpretation of protein denaturation. *Adv. Protein Chem.* 14:1-63.
- Kinsella, J. E., Damodaran, S., and German, B. 1985. Physicochemical and functional properties of oilseed proteins with emphasis on soy proteins. Pages 107-179 in: *New Protein Foods*, Vol. 5. A. M. Altschul, ed. Academic Press: New York.
- Kitabatake, N., Tahara, M., and Doi, E. 1990. Thermal denaturation of soybean protein at low water contents. *Agric. Biol. Chem.* 54:2205-2212.
- Kuntz, Jr., I. D. 1971. Hydration of macromolecules. III. Hydration of polypeptides. *J. Am. Chem. Soc.* 93:514-516.
- Kuntz, Jr., I. D., and Kauzmann, W. 1974. Hydration of proteins and polypeptides. *Adv. Protein Chem.* 28:239-345.
- Lewin, S. 1974. Displacement of water and its control of chemical reactions. Academic Press: New York.
- Marshall, W. E., and Zarins, Z. M. 1989. Exothermic transitions of glycinin determined by differential scanning calorimetry. *J. Agric. Food Chem.* 37:869-873.
- Morales, A., and Kokini, J. L. 1997. Glass transition of soy globulins using differential scanning calorimetry and mechanical spectrometry. *Biotechnol. Prog.* 13:624-629.
- Muffet, D. J., and Snyder, M. E. 1980. Measurement of unfrozen and free water in soy proteins by differential scanning calorimetry. *J. Agric. Food Chem.* 28:1303-1305.
- Nielsen, N. C. 1985. Structure of soy proteins. Pages 27-64 in: *New Protein Foods*, Vol. 5. A. M. Altschul, ed. Academic Press: New York.
- Oates, C. G., Ledward, D. A., and Mitchell, J. R. 1987. Physical and chemical changes resulting from heat treatment soya and soya alginate mixtures. *Carbohydr. Polym.* 7:17-33.
- Oates, C. G., and Ledward, D. A. 1991. Thermal behaviour of soya 7S globulin: Effect of moisture content and added hydrocolloid on the denaturation change in heat capacity. *Food Chem.* 40:101-107.
- Pace, C. N. 1975. The stability of globular proteins. *CRC Crit. Rev. Biochem.* 3:1-43.
- Pradipasena, P., and Rha, C. 1977. Pseudoplastic and rheopectic properties of a globular protein (β -lactoglobulin) solution. *J. Texture Stud.* 8:311-325.
- Privalov, P. L. 1979. Stability of proteins: Small globular proteins. *Adv. Protein Chem.* 33:167-241.
- Privalov, P. L., and Khechinashvili, N. N. 1974. A thermodynamic approach to the problem of stabilization of globular proteins: A calorimetric approach. *J. Mol. Biol.* 86:665-684.
- Roos, Y. H. ed., 1995. *Phase Transitions in Foods*. Academic Press: New York.
- Ross, K. D. 1978. Differential scanning calorimetry of nonfreezable water in solute-macromolecule-water systems. *J. Food Sci.* 43:1812-1815.
- Ruegg, M., Moor, U., and Blanc, B. 1975. Hydration and thermal denaturation of β -lactoglobulin. A calorimetric study. *Biochim. Biophys. Acta* 400:334-342.
- Rupley, J. A., and Careri, G. 1991. Protein hydration and function. *Adv. Protein Chem.* 41:37-172.
- Scilingo, A. A., and Anon, M. C. 1996. Calorimetric study of soybean protein isolates: Effect of calcium and thermal treatments. *J. Agric. Food Chem.* 44:3751-3756.
- Sessa, D. J. 1992. Hydration effects on the thermal stability of proteins in cracked soybeans and defatted soy flour. *Lebensm. Wiss. Technol.* 25:365-370.
- Sessa, D. J. 1993. Thermal denaturation of glycinin as a function of hydration. *J. Am. Oil Chem. Soc.* 70:1279-1284.
- Sheard, P. R., Fellows, A., Ledward, D. A., and Mitchell, J. R. 1986. Macromolecular changes associated with the heat treatment of soya isolate. *J. Food Technol.* 21:55-60.
- Sun, X. S., Kim, H.-R., and Mo, X. 1999. Plastic performance of soybean protein components. *J. Am. Oil Chem. Soc.* 76:117-123.
- Vooijs, A. J., Van Der Steen, P. J., and Beumer, H. 1989. Kinetic methods for research into inactivation of antinutritional factors. Pages 272-275 in: *Recent Advances of Research in Antinutritional Factors in Legume Seeds*. J. Huisman, T. F. B. Van Der Poel, and I. E. Liener, eds. Center for Agricultural Publishing and Documentation: Wageningen, The Netherlands.
- Wright, D. J. 1982. Application of scanning calorimetry to the study of protein behaviour in foods. Pages 61-89 in: *Developments in Food Proteins*, Vol. 1. B. J. F. Hudson, ed. Applied Science: London.

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