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Measurement of Unfrozen and Free Water in Soy Proteins by Differential Scanning Calorimetry

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Soy isolates, soy concentrate, soy flour, and ovalbumin were analyzed for amounts of unfrozen water at different total moisture contents. Analysis was by differential scanning calorimetry to determine heats of fusion and integral heats of vaporization. Unfrozen water did increase with increased total water content. When plotted against total moisture (dry basis), the unfrozen water was a linear function of total water above a concentration of 1 g of water/g of solids, and ~10% of added water was unfrozen. Plots of integral heats of vaporization vs. total water gave peaks below the critical moisture content.

Bound water can be defined as the water that remains unfrozen in samples held well below 0 °C (Fennema, 1977). The amounts of bound (unfrozen) water can be analyzed by differential scanning calorimetry (DSC) (Ross, 1978; Hansen, 1976), by differential thermal analysis (DTA) (Bushuk and Mehrotra, 1977), or by nuclear magnetic resonance spectroscopy (NMR) (Kuntz et al., 1969; Hansen, 1976). The amounts of bound water measured by different methods at low total moisture (below 0.3 g of water/g of solids) generally agree well.

Some analyses of bound (unfrozen) water by DSC and DTA show that the amount of bound water increases as the total moisture content increases above the critical moisture content (Ross, 1978; Bushuk and Mehrotra, 1977; Biswas et al., 1975). There are no NMR studies to our knowledge that show comparable increases above 0.5 g of water bound/g of solids. Hansen (1978) using NMR showed that soy isolates increased in unfrozen water up to 0.5 g/g of solids as total water increased, but the same increase was not found for soy concentrate or for ovalbumin. Conceptually, it is difficult to imagine how primary and multilayer water would continue to increase after the bonding sites are saturated or how capillary water would increase after the capillaries are filled.

We chose to investigate by DSC the water-binding relationships of soy protein as total moisture was increased. We found large increases in unfrozen water as total water increased, but we prefer the term unfrozen to bound water until a suitable explanation for this phenomenon is available.

EXPERIMENTAL SECTION

Reagents. Soy protein isolates were from the Ralston Purina Co. Supro 610 is a hydrolyzed isolate that contains 95% protein and has a pH of 6.7 ± 0.1. Edipro A is 92.5% protein and has a pH of 4.6 ± 0.2. Edipro N is 92.5%

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protein and has a pH of 6.7 ± 0.1.

Promosoy 100, a concentrate with 70% protein and a pH of 7.0 ± 0.1, was from Central Soya Co., Inc. Defatted soy flour, toasted Nutrisoy flour 40, was from Archer Daniels Midland Co. The ovalbumin, salt free and 99% pure, was from Sigma Chemical Co.

Procedures. A Perkin-Elmer differential scanning calorimeter, Model 1B, was used to measure unfreezable water in the protein systems. Each sample, in a volatile sample pan, was cooled to –30 °C and then heated at 20 °C/min to at least 20 °C. Sample size ranged from 2 to 12 mg, depending on moisture content. Samples that contained freezable (free) water produced an endothermic peak, the area of which was measured with a planimeter to determine the total heat of fusion. This was divided by the heat of fusion of pure water, 79.6 cal/g, to determine the weight of freezable water in the sample. This weight was subtracted from the total water to determine the amount of unfreezable water. The total water was determined by drying each sample in its punctured volatile sample pan at 105 °C after completion of the DSC scan.

All weighings were done with a Cahn electrobalance to the nearest 10 μg. The average heat of vaporization of each sample was measured by placing it in a volatile sample pan, sealing, weighing, and then puncturing the lid and placing the sample in a DSC sample holder that had been cooled to 17 °C. The sample was then cooled to 0 °C and scanned to 200 °C at 20 °C/min. All samples produced endothermic peaks, the areas of which gave the total energy needed to vaporize the water. The total energy divided by the weight of water evaporated gave the integral heat of vaporization in calories per gram. Scanning to 200 °C caused all the water to vaporize, and further heating at 105 °C produced no weight change.

Samples that contained less than 40% water were prepared by humidification over distilled water in a vacuum desiccator at room temperature. Water was added directly to produce samples with more than 40% moisture.

RESULTS AND DISCUSSION

Upon measuring the unfrozen water content of Edipro N at different total moisture levels, we found that unfrozen water increased as total water increased. Figure 1 shows
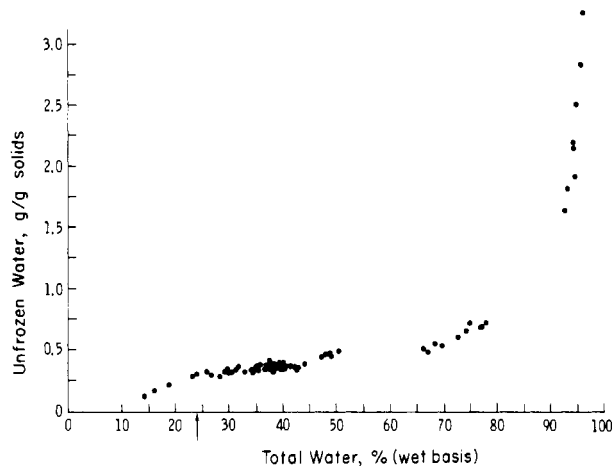
Figure 1. Unfrozen water of Edipro N plotted as a function of total water on a wet basis. The arrow indicates the critical moisture content.
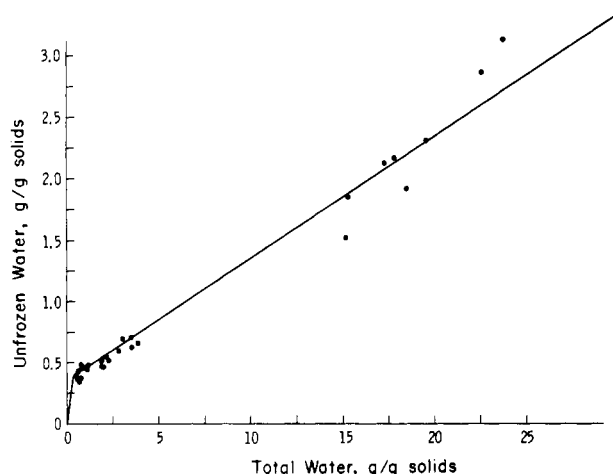

Figure 2. Unfrozen water of Edipro N plotted as a function of total water on a dry basis.

the data for Edipro N over a range of total water contents from 15 to 96% (wet weight). When these data were plotted with both unfrozen water and total water expressed on a dry weight basis, we discovered that there was a linear relationship above the critical moisture content (moisture content at which free water peaks first appear) as shown in Figure 2. The slope of the curve in Figure 2 gives the grams of water unfrozen per gram of total water (numerical value of 0.104). This relationship held in a region of protein concentration of approximately 67% to 4%.

The critical moisture content for Edipro N was 0.32 g of water/g of solids. Between this moisture content and the moisture content at which ~10% of added water becomes unfrozen (a moisture content that we have termed "significant") was a transition zone. In this transition zone, ~50% of added moisture was unfrozen. The significant moisture content for Edipro N was 0.5 g of water/g of solids.

Thus, when unfrozen water per gram of solids was plotted against total water per gram of solids, three regions of the curve were distinguishable. From zero moisture to the critical moisture content, all the water was unfrozen. Between the critical moisture content and the significant moisture content, ~50% of added water was unfrozen, and above the significant moisture content, ~10% of added water was unfrozen. Figure 2 does not show three distinctive regions because the scale was chosen to emphasize the region above 1 g of H_2O/g of solids and not the region between 0.5 and 1 g of H_2O/g of solids.

Table I. Slopes of Unfrozen Water vs. Total Water (Dry Basis) Plots for Several Soy Samples and Ovalbumin

sample	slope	correlation coeff	significance of difference from 0
Edipro N	0.104	0.991	0.005
Edipro A	0.145	0.990	0.005
Supro 610	0.096	0.946	0.01
soy concentrate	0.091	0.988	0.005
soy flour	0.120	0.988	0.05
ovalbumin	0.090	0.986	0.005

Having established that unfrozen water increased as total water increased for one soy protein isolate, we investigated other soy products and ovalbumin. Each substance was analyzed for unfrozen water over a wide range of total water contents, and the data were plotted as in Figure 2. The slopes of the curves (above the significant moisture content) were calculated by using linear regression and are shown in Table I. An F test was used to determine if the slopes were significantly different from zero, correlation coefficients were calculated for each set of data, and these results also are in Table I. All protein products examined showed unfrozen water that increased significantly as total water increased. The amounts of increase were in the range of 0.09–0.14 g of unfrozen water/g of total water.

All the results reported were obtained by the technique given under Procedures. We also experimented with an extended equilibration time (4 °C for 7 or 14 days), a decreased freezing rate, different heating rates, and the times samples were held at −30 °C before heating. None of these changes in procedure had any influence on the measured amount of unfrozen water.

As stated in the introduction, Ross (1978), Bushuk and Mehrotra (1977), and Biswas et al. (1975) all concluded that water binding (unfrozen water measured by DSC or DTA) increaes with increasing water content. Ross (1978) worked with food mixtures as well as with purified casein. The data of Bushuk and Mehrotra (1977) were on wheat flour doughs, and Biswas et al. (1975) studied carbohydrates (mono-, di-, and polysaccharides).

Berlin et al. (1970, 1973) studied whey protein systems by DSC and concluded that bound (unfrozen) water does not change as water increases above the critical moisture content, although the range of water contents studied was small. Ruegg et al. (1974) investigated casein samples by DSC. Their samples varied in water content from 0.3 to 5.0 g of H_2O/g of sample, and they found that bound (unfrozen) water does not vary with total moisture content but presented no data supporting their conclusion.

Duckworth (1971), using DTA, concluded that the water content at which a free water peak first appears is the maximum amount of water bound and that any additional water is free water. However, actual measurements of free and bound (unfrozen) water were not made.

Bushuk and Mehrotra (1977) found that as much as one-third of the added water is bound and suggested that an increased amount of bound water with increasing total water might result from a changed protein configuration that exposes new binding sites.

The moisture-content region in which Bushuk and Mehrotra (1977) found two-thirds of added water free and one-third bound was 0.3–1 g of water/g of solids, the same region in which we found that approximately half the added water was unfrozen.

We investigated the integral heat of vaporization (ΔH_v) of water soy mixtures at various water contents to see if

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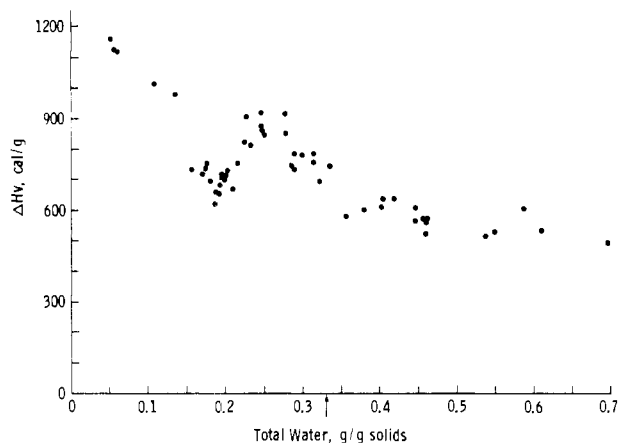*J. Agric. Food Chem.*, Vol. 28, No. 6, 1980   **1305**

Figure 3. Integral heat of vaporization of water from Supro 610 as a function of total water on a dry basis. The arrow indicates the critical moisture content.
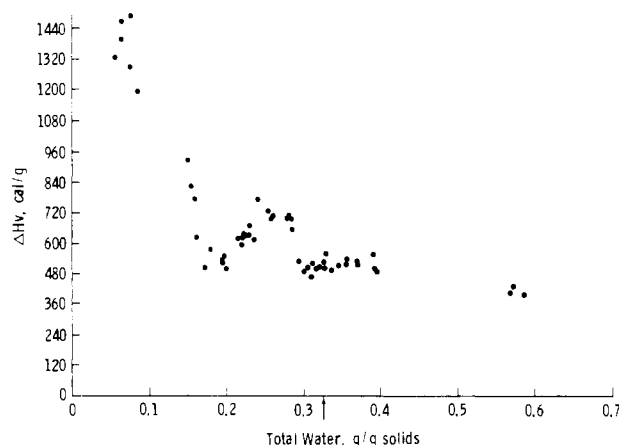

Figure 4. Integral heat of vaporization of water from Edipro N as a function of total water on a dry basis. The arrow indicates the critical moisture content.
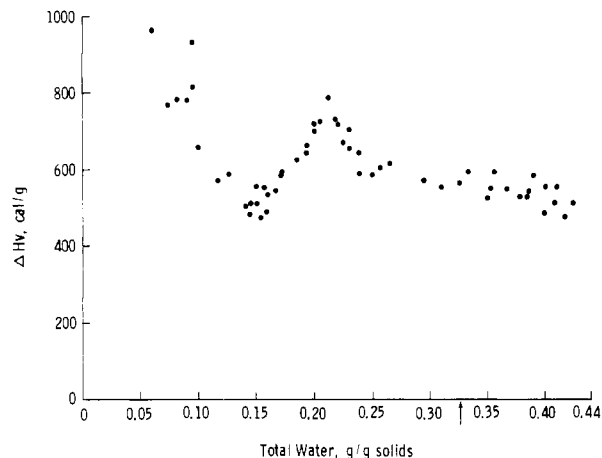

Figure 5. Integral heat of vaporization of water from ovalbumin as a function of total water on a dry basis. The arrow indicates the critical moisture content.

the minimum and the peak for each curve. These changes amounted to 200–230 cal/g, a quantity sufficient to account for an average of one additional hydrogen bond per molecule of water bound.

Bettleheim and Volman (1957) found for some forms of sodium pectate and pectic acid a differential enthalpy of water sorption that had peaks in the region of 0.1–0.2 g of H_2O bound/g of solids. The peaks had a range of ~100–300 cal/g of water—certainly of the same order of magnitude as the ΔH_v peaks we found. They interpreted these peaks as evidence for swelling of pectic acid fibers and exposure of new binding sites. The same interpretation is reasonable for our data.

ACKNOWLEDGMENT

We thank Joan Andersen and Judith Haney for preparation of the manuscript.

Table II. Water Contents and ΔH_v Values Associated with the Peak in Heat of Vaporization

	min point (before increase)		max point		difference in ΔH_v between min and max points, cal/g
	water content, g/g of solids	ΔH_v, cal/g	water content, g/g of solids	ΔH_v, cal/g	
Supro 610	0.19	660	0.25	875	215
Edipro N	0.19	525	0.26	725	200
ovalbumin	0.15	510	0.21	750	230

further information on unfrozen water could be obtained.

Figures 3, 4, and 5 show the ΔH_v as a function of water content for Supro 610, Edipro N, and ovalbumin, respectively. Although the details differ, each curve showed a well-defined peak before the critical moisture content was reached. Table II gives data on the change in ΔH_v between

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