hydrated at 0.98 a_w from dryness. In particular, if the final process with rate constant K_0 represents water transport across soybean cell walls, and K_0 increases with hydration a_w because the cell wall becomes more permeable at higher water contents, then it should be even more permeable in a preequilibrated sample, and hence, K_0 should be larger instead of smaller. The hydration kinetics of partially prehydrated protein is an area in need of further study.

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Hydration of Soybean Protein. 2. Effect of Isolation Method and Various Other Parameters on Hydration

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The state of water in various soybean protein preparations is characterized by NMR determination of water-binding capacity and sorption isotherm measurement of total water sorption. Hydration states are compared for proteins isolated by different methods, as a function of heat, total water content, particle size, and ultrasonic irradiation. Hydration properties of samples prepared by adsorption and desorption methods are compared and found to be similar. Some speculation is made regarding the causes of the changes in hydration state resulting from the above treatments.

In a previous publication (Hansen, 1976), a model was developed for the hydration state of soybean proteins. This model was based on: (1) water sorption isotherm data at three temperatures, from which thermodynamic functions, BET (Brunauer et al., 1938) and Bradley (1936) parameters were derived; and (2) nuclear magnetic resonance (NMR) measurements of water proton nuclear spin relaxation times and unfrozen water content vs. temperature. The results of these various methods for characterizing the state of water in soy protein concentrate are summarized in the following statements:

(1) Water present up to ~0.07 g of water/g of solids is "tightly bound" (BET monolayer) and probably is water of hydration of ionic protein binding sites.

(2) Water present above the "monolayer" value up to ~0.25 g of water/g of solids is more "loosely bound", probably water associated with polar protein and carbohydrate groups and/or secondary water of hydration of the "tightly bound" hydration groups. There is a wide distribution of molecular mobilities for this water species, presumably reflecting a distribution of binding energies for water.

(3) Water present above the "loosely bound" level is more like bulk liquid, or "free" water, in terms of its molecular mobility and freezing pattern.

In this paper, some speculations were also made about the effects of food hydration state on rates of food degradation processes such as lipid oxidation, nonenzymatic browning, and microbial growth.

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Since food proteins, especially those derived from soybeans, may undergo a variety of treatments during isolation and processing, it seemed important to determine the effects on water binding of some of these treatments (e.g., heat), using the previously developed techniques. For this reason the effect of protein type and content (e.g., soy flour, concentrate, isolate), particle size, heat "denaturation" in the presence of water, oven-drying, total water content, disruption of remaining soybean cell structure by ultrasonic irradiation, and hydration methods (sorption vs. desorption) on protein hydration state, as measured by sorption isotherms and/or NMR measurements of water binding, have been determined.

EXPERIMENTAL SECTION

Materials. The soy protein concentrate was the same material used in the previous study (Hansen, 1976), prepared by repeated extraction of defatted soybean flakes with 70% ethanol, followed by 100% ethanol extraction, and air-drying at 35 °C. Ovalbumin (lipid-free, 3 × recrystallized) was from Worthington Biochemicals. The ACP-950 soy protein isolate was obtained from Anderson Clayton Foods, Inc., Dallas, Tex. The 7S soy protein isolate was prepared by dispersing defatted soy flakes in water (1 g of solids/9 g of water), extracting protein after adjustment of the pH to 8.6, followed by centrifugation to remove solids. The supernatant was then adjusted to pH 4.5 to precipitate what is predominantly a 7S protein. The pH of the precipitate was adjusted to 7 and the material freeze-dried. The soy protein isolate A was prepared from soy concentrate by standard methods (Wolf and Cowan, 1971). Soy isolate B was prepared as A, except rather than precipitating the protein by adjustment of pH

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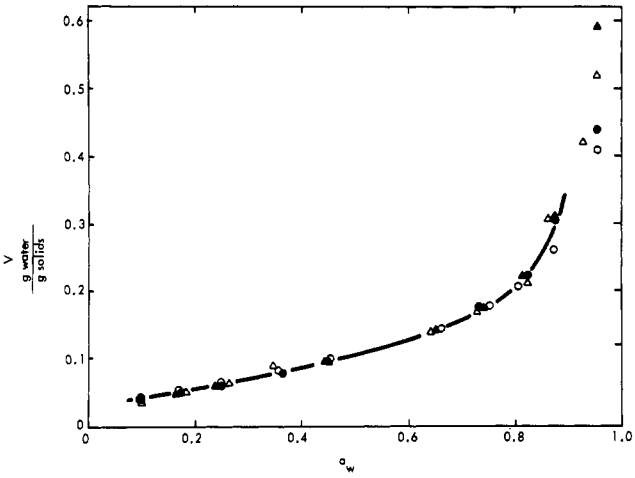

Figure 1. Water sorption isotherms for three protein isolates and soy concentrate at 37 °C: (△) soy protein isolate A, (○) soy protein isolate B, (▲) ACP-950 soy isolate, (●) soy protein concentrate.

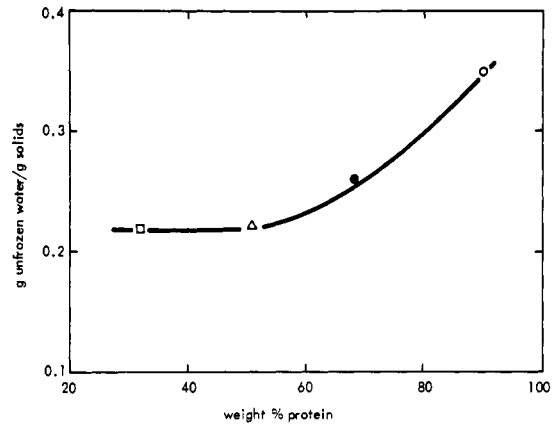

Figure 2. Water binding (g of unfrozen water/g of solids) by soy protein preparations, each containing 1 g of water/g of solids, as a function of protein content, where % protein (on a solids basis) = % N × 6.25: (○) soy protein isolate B, (●) soy protein concentrate, (△) soy flour (defatted), (□) carbohydrate-enriched fraction of soy concentrate.

to 4.5, precipitation was accomplished at pH 7 by addition of ethanol to give a 50% aqueous solution, followed by centrifugation and washing with 50% ethanol solution. Protein contents were determined by Kjeldahl analysis using % protein = % N × 6.25.

Methods. Sorption isotherms were measured at 21 °C by the previously reported method (Hansen, 1976). NMR measurements were also performed as previously reported (Hansen, 1976). The water-binding capacity of the materials is defined as the unfrozen water content (in g of water/g of solids) of the hydrated sample at −50 °C, as measured by NMR (Kuntz, 1971; Hansen, 1976). Soy concentrate was screened to obtain different particle size ranges by mechanical shaking of a stack of different mesh size screens for 30 min. Samples were heat "denatured" by heating the hydrated protein in sealed 12-mm diameter test tubes in a boiling water bath for 20 min. Ultrasonic irradiation was at 20 kHz for 20 min, using the full-power setting of a Branson Model L sonifier converter and S-12a power supply with the sample surrounded by an ice bath to prevent heating.

Desorption isotherms were determined by directly mixing the solid material and water together to give a 2:1 weight ratio of water to solids and then placing the samples in vacuum desiccators over sulfuric acid solutions of known water activities. The remainder of the procedure was similar to that previously reported for sorption isotherms (Hansen, 1976).

RESULTS AND DISCUSSION

The effects of the procedure used to prepare the proteinaceous material and of protein content on hydration are shown in Figures 1 and 2. Figure 1 shows sorption isotherms at 37 °C for three protein isolates (ACP-950 and isolates A & B) and soy protein concentrate. At water activities (a_w) below ~0.85 the various isotherms are identical within experimental error. Above ~0.85 a_w there are some differences in water sorption vs. a_w, the trend being ACP-950 isolate > soy protein isolate A > soy concentrate ≳ soy isolate B. Figure 2 shows the NMR-determined water binding data (g of water unfrozen at −50 °C/g of solids) for four soybean protein samples of different preparation/isolation procedure and different protein contents (soy protein isolate B, 90% protein; soy concentrate, 70% protein; soy flour, 50% protein; and a carbohydrate-enriched soy fraction, 32% protein). A general trend of increased water binding with increased protein content is seen. However the increase is not linear with protein content, as is evident from Figure 2, suggesting that factors in addition to protein content contribute to water binding.

There is an apparent discrepancy between the trends observed in the water *sorption* isotherm data and the NMR-determined water *binding* data, e.g., soy concentrate sorbs slightly more water than the soy isolate B at constant a_w above ~0.85 and ACP-950 sorbs considerably more than either of the above, while the water binding results are in the order soy concentrate < ACP-950 < soy isolate B. In addition, for $a_w \lesssim 0.85$ (≲0.25 g of water/g of solids), water sorption values are similar for the three materials, despite their different measured water-binding capacities. This apparent discrepancy can be resolved to give additional insight into the hydration model for these materials: (1) in the water content region below ~0.25 g/g, where all (or most) of the water is associated with the solid protein ("bound"), the a_w of the system is determined by the nature of the water–protein interaction, and this is similar for the different materials studied (hence similar isotherms). (2) Above ~0.25 g/g, where some "free" water begins to appear, the a_w of the system is determined in part by the water binding characteristics, but also by the solubility and molecular weight distribution of the protein and carbohydrate components of the soy protein preparations (i.e., an osmotic effect) such that materials with higher solubility and lower average molecular weight will sorb more water at a given a_w.

Figure 3 illustrates the effect of total water content on water binding (unfrozen water content) by soybean proteins. Data for ovalbumin are also included as an example of an animal protein. Two very different types of water-binding behavior with total water content are seen. In the case of soy protein concentrate and ovalbumin, the water-binding capacity is invariant with total water content from ~0.3 to ~2.0 g of water/g of solids. Soy protein isolates A and B, on the other hand, more than double in water-binding capacity between 0.3 and 3 g of water/g of solids total water contents. This increase in water binding could be due to a swelling of the hydrated protein matrix and/or to a protein conformation change with increasing water content, which leads to exposure of additional water binding sites. This change is reversible, as evidenced by the lack of hysteresis in either the sorption isotherm or the NMR-determined water-binding capacity (discussed later). The soy proteins A and B, isolated by different methods,

Table I. Effect of Total Water Content, Heating (in the Presence of Water), and Oven-Drying on the Water-Binding Capacity of Several Proteins

Sample	g of total water/g of solids	g of unfrozen ("bound") water/g of solids, measured at 25 °C		
		"Native"	Heated 100 °C	Dried 100 °C/rehydrated
Soy protein concentrate	0.70	0.25	0.25	0.29
	0.96			0.26
	2.0	0.26	0.27	0.35
Sonicated soy concentrate	1.90	0.33	0.43	0.28
Soy isolate A	0.65	0.32		
	2.0	0.45		
Soy isolate B	0.96	0.35		0.33
	1.9	0.46	0.40	0.39
	2.3	0.47	0.38	
7S soy isolate	0.93	0.28	0.26	
ACP-950 soy isolate	2.0	0.36		
Ovalbumin	0.33	0.33		
	1.0	0.33	0.39	0.32

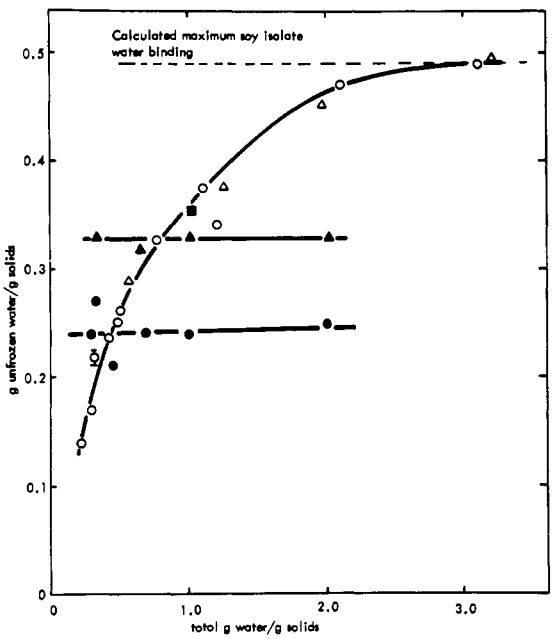

Figure 3. Unfrozen ("bound") water content as a function of total water content for several proteins. The dashed line indicates maximum water binding capacity calculated for soy protein isolate: (△) soy protein isolate A, (○) soy protein isolate B, (■) soy protein isolate B hydrated by desorption technique, (●) soy protein concentrate, (▲) ovalbumin.

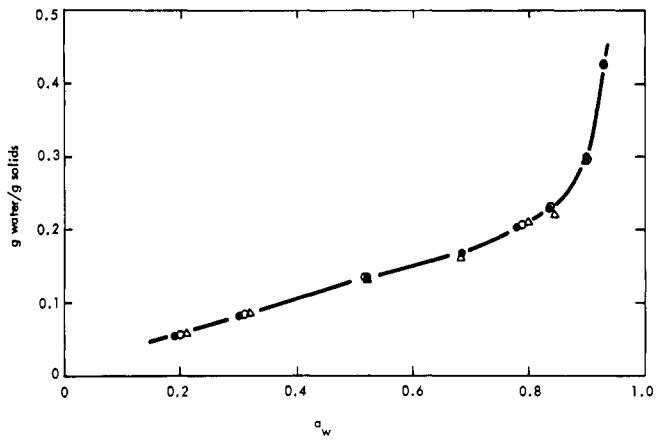

Figure 4. Water sorption isotherms at 21 °C for soy protein concentrate: (1) screened to particle size ranges 350–420 μm (○) and 44–74 μm (●), (2) irradiated with ultrasonic energy (△).

are seen to have essentially identical water-binding properties. Note that at high water contents, the observed water binding for soy isolates A and B approaches the value (0.49 g/g) calculated from the water-binding abilities of the individual amino acid residues, according to the method of Kuntz (1971), implying full hydration of all water-binding sites. This behavior is in contrast to that observed for ovalbumin (discussed later), in which the maximum hydration is attained only after heating.

The increase in unfrozen (bound) water content with increasing total water content found for soy protein isolates is similar to that found for human stratum corneum, as determined by infrared spectroscopy (Hansen and Yellin, 1972). Here the infrared band attributed to water associated with polar protein binding sites was found to increase in relative intensity as the total water content increased, indicative of a protein conformation change to expose more water-binding sites upon increased hydration. The absence of this behavior in the case of soy protein concentrate may be due to the presence of the soybean cellular structure, which inhibits swelling of the hydrated protein. In contrast, this cell structure is absent in the protein isolates, the cells having been disrupted and the bulk of the cell wall material removed. This view is supported by the increased water binding (unfrozen water content) found at high total water content for soy concentrate whose cell structure has been largely disrupted by sonification (Table I). It is not known why the water binding of ovalbumin does not increase with increasing total water content, as with the soy protein isolates. However, this may be the result of a more stable conformation in the case of ovalbumin, due in part to intramolecular disulfide bonds, which is not changed to expose more water-binding sites except upon heating.

The effect of particle size of the solid protein on hydration was determined by measurement of water sorption and NMR water-binding data for two soy protein concentrate fractions screened to give particle diameter ranges of 44–74 and 350–420 μm. That water sorption over the a_w range 0.15 to 0.95 is invariant with particle size is evident from the sorption isotherms of these two materials at 21 °C, which were identical within ≤2% (Figure 4). The BET "monolayer" hydration value calculated from these sorption isotherms is 0.072 ± 0.002 g of water/g of solids, for both samples. Water-binding capacities determined from NMR measurements on samples containing 1.0 g of water/g of solids are 0.27 and 0.28 g of bound water/g of solids for the 350–420 and 44–74 μm size ranges, respectively. These findings are consistent with the site-binding hydration model in which the "bound" water species are assumed to be associated with specific amino acid residues rather than surface-adsorbed.

Table II. Adsorption (V_a) and Desorption (V_d) Isotherms at 37 °C for Soy Protein Isolate B and Concentrate

Sample	a_w	g of water/g of solids	
		V_a	V_d
Soy isolate B	0.17	0.055	0.070
	0.955	0.435	0.440
	0.965	0.438	0.448
Soy concentrate	0.17	0.063	0.070
	0.31	0.090	0.100
	0.52	0.115	0.115
	0.68	0.150	0.150
	0.79	0.190	0.180
	0.84	0.210	0.210
	0.90	0.290	0.290
	0.93	0.440	0.445

Table I shows how heat "denaturation" (100 °C) changes the water-binding capacities of several soybean protein preparations and ovalbumin. These measurements were all made at 25 °C on samples with prior heat treatment. The water-binding capacity of ovalbumin increases by ~20% upon heating, while that of soy protein concentrate and 7S isolate is virtually unchanged with heat. Water binding by soy isolate B and ACP-950 isolates decrease and increase slightly, respectively, upon heating.

The unfrozen water contents for native ovalbumin are identical with that found by Kuntz (1971) at a much higher total water content. The increase after heat denaturation is presumably the result of protein unfolding to expose more water-binding sites, as postulated by Kuntz (1971) to explain the ~10% increase in unfrozen water content upon urea denaturation of bovine serum albumin. In general, the water binding by soy proteins either does not change much or decreases upon heat "denaturation" (sonicated soy concentrate being an exception). The explanation for this apparent inconsistency (unfolding upon heating should expose more water-binding sites) probably lies in the fact that these proteins form thermally irreversible gels upon heating and cooling. This process can be envisioned as an unfolding of protein (perhaps accompanied by disaggregation of subunits, in the case of soy proteins) followed by intermolecular interactions of whatever type (disulfide, ionic, hydrogen, "hydrophobic"?) are responsible for gel formation. In the case of ovalbumin, this process does not result in blockage of any water-binding sites. In fact, the observed unfrozen water content after heating (0.39 g/g) is the same, within experimental error, as that calculated (0.37 g/g) for the completely unfolded protein, based on the water binding capacities of the individual amino acid residues (Kuntz, 1971). Thus, in the case of ovalbumin, thermal gelation does not involve the water binding sites (except for the possibility of protein–protein interactions via water bridges). In the case of the soy proteins, the gelation process apparently results in decreased water-binding capacity. This could be the result of formation of protein–protein hydrogen bonds from what were previously protein–water bonds. In contrast to ovalbumin, gelation in soy protein does appear to involve water binding sites.

Ultrasonication increased the water-binding capacity as shown in Table I. In addition, the water-binding capacity of sonicated soy concentrate was rather greatly increased upon heating at 100 °C, while the unsonicated material was unchanged in water binding by heat. Ultrasonic irradiation of soy concentrate completely breaks up the soybean cell structure and the observed increase in water-binding capacity both before and after heating could be due to increased swelling of the hydrated protein matrix. The increase in water binding could also be due to a change in protein conformation or aggregation caused by the ultrasonic energy, although the ultracentrifuge sedimentation velocity pattern of the soluble protein is unchanged by sonication, indicating that subunit aggregation is unchanged for the soluble protein.

Some light is shed on the question of whether a real difference exists between adsorption and desorption isotherms (equilibration of samples which are initially below and above their equilibrium water content, respectively, with water vapor of known activities) by the data shown on Table II and Figure 3. Table II shows water contents of pairs of soy protein isolate B and soy concentrate samples (one initially dry; one initially containing 2 g of water/g of solids) after being placed in vacuum desiccators containing aqueous sulfuric acid solutions at various a_w for 40 days at 37 °C. No significant differences between water sorption values for ad- and desorption techniques are found. Differences could be observed at shorter "equilibration" times, however. The rate of water loss by the initially hydrated samples after being placed in the constant a_w chambers was characterized by a relatively rapid initial loss of water, followed by a second, much slower dehydration phase, which lasted for several weeks. Water sorption–desorption "hysteresis" is thus a kinetic rather than an equilibrium phenomenon in these materials. This conclusion is further supported by NMR measurement of unfrozen water content of two soy isolate B samples, one hydrated from dryness to 1 g of water/g of solids; one dehydrated from 3 to 1 g of water/g of solids. These samples give identical unfrozen ("bound") water contents, as shown on Figure 3.

This study shows that the isolation method and a number of treatments to which soybean proteins might be subjected in their use in foods can change their water-binding properties. It is also shown that measurement of the water-binding capacity of proteins (e.g., by NMR) is more sensitive to these changes than total water sorption measurements at various a_w. These measurements by themselves are not sufficient to completely determine what molecular changes are responsible for the observed changes in hydration, but do suggest some possibilities.

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