Hydration of Soybean Protein

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The state of water in a soybean protein concentrate was characterized by a combination of nuclear magnetic resonance (NMR) and sorption isotherm measurements. The association of water with soy protein is described as a "site-binding" phenomenon. Three types or species of water can be distinguished, each having a different dependence of molecular reorientation rate ("mobility") on water content and/or different freezing behavior. A similar situation is also found for the animal-derived protein, ovalbumin. From a comparison of these measured hydration states of soy protein with data from the literature on rates of various food degradation processes (e.g., lipid oxidation, nonenzymatic browning, bacterial growth) as a function of water activity, some speculations are made about the effects of hydration state on these processes.

A number of processes which lead to the degradation of foodstuffs, such as microbial growth, lipid oxidation, nonenzymatic browning, and enzyme activity, are known to depend upon the water content and water activity of the food (Mossel and Ingram, 1955; Morris and Wood, 1956; Rockland, 1957; Scott, 1957; Labuza et al., 1970, 1972a,b; Bone, 1973; Karel, 1975). It is clear from these reports that the hydration state of a food, as measured by water activity, is an important parameter in determining food stability. There is also some evidence that water content, as well as water activity, may be important in determining the microbial stability of foods. It has been reported (Labuza et al., 1972a; Plitman et al., 1973) that microbial growth was higher in meat and banana intermediate moisture content foods adjusted to a particular water activity by a desorption technique than in the same food adjusted to the same water activity (but lower resulting water content) by adsorption of water from the dry state. The simple fact that water, which is necessary for the growth of microorganisms, need not be completely removed from a food to prevent spoilage suggests that there is a relationship between the state of water in a food and its ability to support microbial growth.

Several studies aimed at characterizing the nature of water in foods and model food systems have been reported (Rockland, 1969; Duckworth, 1971, 1975; Shanbhag et al., 1970; Duff and Derbyshire, 1974; Karel, 1975; Cooke and Kuntz, 1974; Kuntz and Kauzman, 1974). Okamura (1973) reported high-resolution proton nuclear magnetic resonance (NMR) spectra of soy flour. The resonance due to water was identified and its line width found to decrease with increasing water content. This behavior was interpreted as arising from breaking of hydrogen bonds, although the type of hydrogen bond (e.g., water–water or protein–water) was not specified.

The purposes of the present work are to characterize the state of water in hydrated food proteins, compare the hydration of soybean protein and ovalbumin, and examine the possible relationship between water binding by protein and food degradation processes (e.g., microbial growth). Soybean protein was chosen as the basic model food system because of (1) its increasing importance as a protein source, and (2) its relative simplicity and reproducibility in preparation and handling, compared to whole foods such as meats. Ovalbumin was also included in the study to provide a comparison between plant and animal-derived proteins.

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Because of the utility of NMR in determining the state of water in protein–carbohydrate systems, two NMR techniques were used in the present study of soybean protein hydration. These two techniques measure the amount of water in a hydrated sample which does not freeze (presumably due to sufficiently strong water–protein interactions to prevent water molecules from adopting the ice structure), and the average water molecular mobility (also a function of water binding) as a function of total water content. Water sorption isotherms were also measured to allow comparison of the data with several sorption models, and to make possible a comparison of the NMR water-binding data with food stability data from the literature.

EXPERIMENTAL SECTION

Nuclear Magnetic Resonance. Two nuclear magnetic resonance methods were used to probe the state of water in soy protein. (1) The first method is measurement of the nuclear spin relaxation time of water in hydrated protein samples as a function of water content. This relaxation time is proportional to the molecular mobility of water molecules (more precisely, to the inverse of the correlation time describing molecular motion, which for rotation can be considered as the time required for rotation through ~1 radian). If there are several species of water with different mobilities present in the sample and exchange of molecules between sites is sufficiently rapid, then an exponential relaxation curve will be observed. The time constant for this relaxation will be the weighted average of the relaxation times for the various water species. The relative amounts of each water species can be deduced from the water content dependence of the observed relaxation time. (2) The second method is a comparison of the amplitude of the NMR water signal at temperatures above and below 0 °C. Above 0 °C the signal amplitude is proportional to the total water content of the sample. Below 0 °C, that fraction of the total water which interacts so weakly with the protein that it can adopt the ice structure, freezes. Since our spectrometer does not respond to NMR signals from ice, the NMR signal amplitude at some low temperature (e.g., −50 °C) represents the water species which interacts so strongly with the protein that it cannot freeze. Signal amplitudes were measured by extrapolation of the free induction decay back to the end of the 90° pulse, to take into account the ~15 μs dead time of the spectrometer receiver. This correction becomes more important at lower temperatures, where the free induction decay becomes quite rapid.

Because the signal amplitude can change with temperature even in a sample containing a fixed amount of

liquid water, the instrument had to be "calibrated" to correct for these changes. These signal intensity changes with temperature could be due to changes in the equilibrium spin state populations or to changes in the NMR probe response. The proper correction was determined using the method reported by Fung (Fung and McGaughy, 1974). This corrected water signal amplitude then represents (after normalizing by setting the average of all the amplitudes above 0 °C equal to 1.0) the fraction of the total water in the sample which is not frozen at the temperature of the measurement. Both of these methods should provide a measure of the interaction of water with the protein, the first being a measure of molecular "mobility", and the second a measure of "order" of hydrogen-bonding "structure".

A Magnion ELH-30 pulsed NMR spectrometer with resonance frequency 36 MHz was used for the measurements. For measurement of relaxation times T_2 less than ~1 ms, the free induction decay after a 90° pulse (Carr and Purcell, 1954) was measured, and for longer relaxation times, the Carr–Purcell pulse sequence with Meiboom–Gill (1958) modification was used.

Adsorption Isotherms. Adsorption isotherms (water content vs. water activity) were measured by placing weighted amounts of sample, which had previously been dried under vacuum over calcium sulfate for 48 h, over various aqueous sulfuric acid solutions of known water activity. Sulfuric acid solutions were made up volumetrically, and their composition, from which water activity was determined (Washburn, 1928), was measured by titration before and after each adsorption isotherm measurement. Sulfuric acid solutions were used for the 1, 21, and 37 °C isotherms, and as a check, aqueous salt solutions (Young, 1967) were also used at 21 °C. The samples and solutions were placed in vacuum desiccators which were evacuated until the solutions just began to boil. The desiccators were then placed in a controlled environment chamber (an incubator for the 37 °C runs, and controlled temperature rooms for the 1 and 21 °C runs), in which the temperature was controlled to ±1 °C. In most cases, this technique allowed equilibration of the solution and the sample within 48 h for $a_w < 0.90$ and ~7 days for $a_w \geq 0.90$, without sample spoilage. When equilibrium had been established, as determined by constant sample weight, the water content at each a_w value was determined gravimetrically to ±0.0001 g by vacuum oven moisture determination (85 °C, ~50 mmHg, 48 h). Samples of approximately 1 g size were used. Desorption isotherms at 21 °C were determined by equilibration of samples containing excess water (~1 g of water/g of solids) with sulfuric acid solutions, as described above. Final water contents were determined by vacuum oven drying.

Materials. The soy concentrate used in this study was prepared as follows: full-fat soy flakes were extracted with hexane. The protein concentrate was prepared by eight extractions of these flakes with a 70% ethanol solution (aqueous), followed by extraction with 100% ethanol. The resulting concentrate was dried in air at 35 °C, followed by vacuum drying. The material is 69% protein (% N × 6.25), 6.1% ash, and 0.4% lipid, on a dry weight basis. Ovalbumin was lipid free, three times recrystallized, lyophilized material obtained from Worthington Biochemicals.

RESULTS AND DISCUSSION

Adsorption Isotherms. Adsorption isotherms for soy concentrate and ovalbumin at 21 °C are shown in Figure 1. For soy concentrate, adsorption isotherms were also measured at 1 and 37 °C and the desorption isotherm was

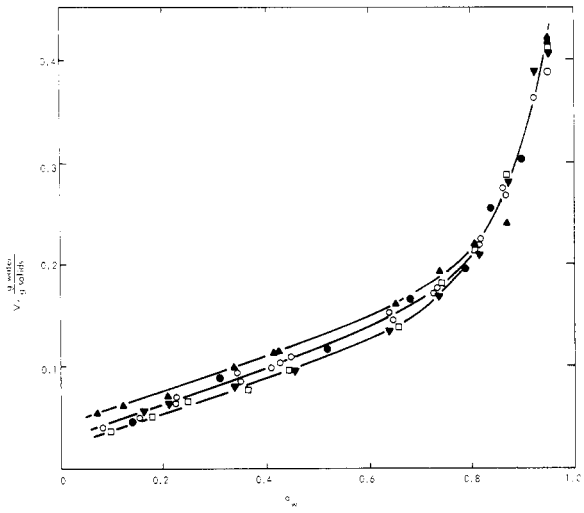

Figure 1. Water adsorption isotherms at several temperatures and desorption isotherm at 21 °C for soy protein concentrate and at 21 °C for ovalbumin: (▲) soy protein concentrate, 1 °C adsorption; (○) soy protein concentrate, 21 °C adsorption; (●) soy protein concentrate, 21 °C desorption; (□) soy protein concentrate, 37 °C adsorption; (▼) ovalbumin, 21 °C adsorption.

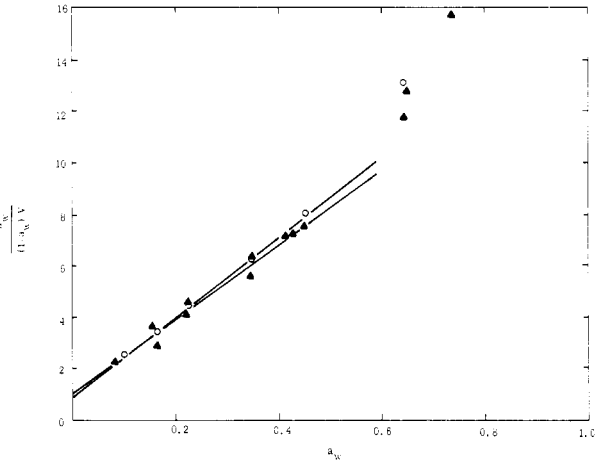

Figure 2. BET plots for soy protein concentrate, from 21 °C water sorption isotherm data: (▲) soy protein concentrate; (○) ovalbumin.

measured at 21 °C for the a_w range 0.15 to 0.93. No differences were found between adsorption and desorption isotherms, within the experimental error of ±5%, although equilibration times were considerably longer for the desorption samples (7–28 days, depending upon a_w). This result is in contrast to data reported on some pure proteins (McLaren and Rowen, 1951; Altman and Benson, 1960) and whole foods (Labuza et al., 1972a; Plitman et al., 1973; Chou et al., 1973) and indicates that a true equilibrium hydration state is reached in these soy proteins if long enough equilibration times are allowed. The 21 °C results are in good agreement with those obtained by Okamura (1973) on whole soybeans and by Saravacos (1969) on defatted soybeans.

The Brunauer, Emmett, and Teller (BET) equation (1938) is often used to characterize water sorption by food materials, including proteins and carbohydrates (Salwin, 1959; Labuza et al., 1970). The interpretation of the BET parameters to describe macromolecular hydration is discussed by Ling (1972). Figure 2 is a BET plot of the water adsorption data for soy protein concentrate and ovalbumin. The "monolayer" hydration values, V_m, and

Table I. Summary of Water Binding Data Obtained by Several Methods for Soy Protein Concentrate and Ovalbumin

		"Bound" water, g of water/g of solids			
	E_1,[a] kcal/mol	V_m, BET "monolayer"	Bradley[b] isotherm	NMR unfrozen[c] water at −50 °C	NMR molecular[d] mobility (from T_2)
Soy protein concentrate	12.1	0.064 ± 0.002	0.26	0.26 ± 0.03 (1.0) 0.26 ± 0.03 (2.0)	0.26
Ovalbumin	12.3	0.061 ± 0.004	~0.15	0.33 ± 0.03 (1.0) 0.33 ± 0.03 (2.0)	0.20

[a] Heat of sorption of water "monolayer", calculated from BET equation and isotherm data at 21 °C. [b] "Multilayer" water contents, calculated from the Bradley equation and sorption isotherm data at 21 °C. [c] Grams of unfrozen ("bound") water/gram of solids at −50 °C, determined by NMR, measured at total water content indicated in parentheses. [d] Values calculated from the breakpoints in T_2^{-1} vs. V^{-1} plots at 21 °C.

the heat of adsorption of the monolayer water, E_1, calculated from these data at 21 °C are shown in Table I. The BET monolayer adsorption values for ovalbumin and soy protein are similar to those reported by Bull (1944) for serum albumin, egg albumin, and a number of other proteins, and slightly higher than the value (0.0546 g/g) found by Okamura (1973) for whole soybeans. It should be noted that although the data fit the BET equation reasonably well at low water activities, the derived monolayer values probably represent water associated with the most hydrophilic amino acid residues ("site-bound" water), rather than a true monolayer of surface-adsorbed water, a proposition first put forth by Pauling (1945). The "surface area" calculated from these monolayer values is ~200 m^2/g, which is much lower than that estimated for complete surface coverage of the (assumed spherical) protein molecules. If association of one water molecule per ionic amino acid residue is assumed for the initial hydration, a value of 0.066 g of water/g of protein is calculated, based on the known amino acid composition (Rackis et al., 1961) of soy protein. This hydration value is very similar to the BET monolayer values found, and supports the water site-binding hypothesis.

The lack of fit of the experimental water adsorption values to the BET equation at $a_w \gtrsim 0.5$ is typical (see, e.g., Ling, 1972), and could be due to "multilayer" sorption of water (filling of additional water binding sites) with successive layers having different heats of sorption. The fit of the experimental data for a multilayer sorption equation was also tested. Bradley (1936) derived an equation to describe the sorption of molecules possessing a permanent dipole moment onto ionic surfaces (an extension of an earlier theory of DeBoer and Zwikker (1929)). Bradley's sorption equation is based on the assumption that the first layer of molecules is sorbed with a net orientation of dipole moments induced by the polar surface. This adsorbed ("polarized") layer in turn polarizes another layer, which polarizes a third, etc. In this way, a "polarized multilayer" of molecules is built up on the surface. The equation derived by Bradley is, in linear form:

$$\log (\log (1/a)) = \log K_2 + V \log K_1$$

Here a and V are the activity and amount of vapor sorbed, respectively, and K_1 and K_2 are constants which are functions of the dipole moment of the sorbed vapor and of the sorptive polar groups, respectively. One would thus expect a system exhibiting multilayer sorption to give a linear plot of log (log $(1/a)$) vs. V. The Bradley equation has been applied to sorption of water by silk and polyglycine with some success (Hoover and Mellon, 1950).

Figure 3 shows Bradley isotherm plots of the water sorption data at 21 °C. The plots are linear up to water contents of ~0.15 to 0.25 g of water/g of solids for

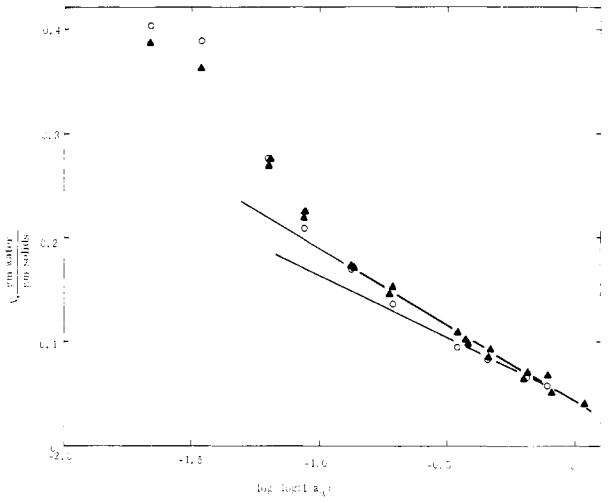

Figure 3. Bradley isotherm plots for water sorption at 21 °C by soy protein concentrate and ovalbumin: (▲) soy protein concentrate; (○) ovalbumin.

ovalbumin and soy concentrate, respectively. Above this range of water contents (corresponding to water activities of ~0.70 to 0.85), the experimental points diverge from linearity. This behavior is consistent with multilayer sorption of water for water contents up to ~0.15–0.25 g/g. The maximum amounts of multilayer water sorbed, as determined from the Bradley plots for proteins, are given in Table I.

Agreement of the experimental water sorption data with the isotherm equation calculated for a particular sorption model does not, of course, provide unambiguous proof that the particular sorption model assumed is correct, since the agreement may not be unique.

Thermodynamic Parameters. The thermodynamic parameters describing the sorption of water on proteins may also be calculated from the sorption isotherm data. The differential free-energy, enthalpy, and entropy changes for the sorption process (referred to pure liquid water) were calculated by standard methods (Bettleheim and Volman, 1957; Adamson, 1967), the differential (isoteric) enthalpy being determined from the sorption isotherms at three temperatures, using the Clausius–Clapeyron equation. These differential thermodynamic quantities are plotted as a function of water content on Figure 4. Values of $\Delta\bar{H}$ calculated from Bull's (1944) data on water sorption by ovalbumin are also shown on Figure 4 for comparison.

The differential thermodynamic functions calculated from the water sorption isotherms also support the notion of multilayer (or multibinding site energies) sorption. At low water contents the differential free energy is a relatively large negative number, decreasing rapidly in absolute value with increasing water content up to ~0.2 g of water/g of solids. The differential enthalpies and entropies

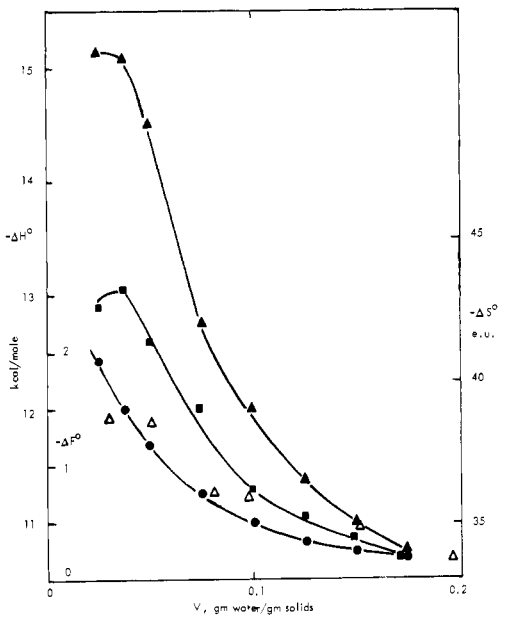

Figure 4. Differential enthalpy, free energy, and entropy functions at various water contents for soy protein concentrate and ovalbumin (ΔF and ΔS calculated at 27 °C). The ovalbumin results were calculated from Bull's (1944) data. Soy protein concentrate: (▲) $-\Delta H°$; (●) $-\Delta F°$; (■) $-\Delta S°$. Ovalbumin (from Bull's (1944) data): (△) $-\Delta H°$.

also have large negative values at low water contents, being approximately constant up to water contents of ~0.05 g/g (although the errors in these functions are quite large at these lower water contents, ~±20%), and then decreasing rapidly in absolute value above ~0.05 g/g. This phenomenon may reflect filling of the water binding sites corresponding to the calculated BET monolayer (~0.06 g of solids). In addition, the apparent maximum in $-\Delta S$ at ~0.04 g/g may reflect exposure of more water binding sites as water is adsorbed, as found for water sorption by pectins (Bettelheim and Volman, 1957), although the errors in ΔS are large enough to make this conclusion speculative. Above ~0.05 g/g, the differential enthalpy decreases rapidly in absolute value with increasing water content, indicating decreasing water binding energetics. The overall dependence on water content found for the thermodynamic functions for water sorption by these proteins is rather similar to that reported for water sorption by certain pectins (Bettelheim and Volman, 1957). As has been pointed out by these and other authors, differential thermodynamic functions calculated from water sorption isotherms may represent thermodynamic changes due not only to sorption of water by the polymers, but also due to changes in polymer configuration (e.g., swelling). For this reason, these thermodynamic data alone are not sufficient for determining a model of water sorption by soy proteins, but do serve as an additional check on any model assumed.

NMR Results. The NMR data can provide independent and relatively direct evidence on the state of water in these soy proteins as a function of water content and activity, giving a more definitive picture of hydration. The two NMR methods used here provide information on molecular mobility as a function of water content, and on water "structure" (ability of water to freeze) in the presence of protein.

One way of measuring water binding is to measure the NMR relaxation time, T_2, which is proportional to the water molecular mobility, as a function of water content. The equations describing the spin–spin relaxation time, T_2, of water protons in the case of rapid exchange between

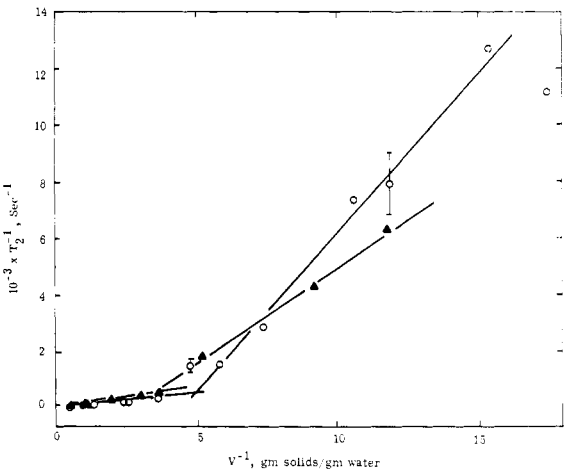

Figure 5. Water proton spin–spin relaxation rate (T_2^{-1}) as a function of protein concentration in water at 25 °C for soy protein concentrate and ovalbumin. The standard deviation for each value, based on replicate measurements on the same sample, is indicated: (▲) soy protein concentrate; (○) ovalbumin.

"free" and "bound" water are:

$$T_2^{-1} = \frac{V_b}{V}(T_{2b}^{-1} - T_{2f}^{-1}) - T_{2f}^{-1} \text{ for } V > V_b$$

$$T_2^{-1} = T_{2b}^{-1} \text{ for } V \leq V_b$$

where V_b and V are grams of bound water and grams of total water per gram of solids, respectively, and T_{2b} and T_{2f} are the relaxation times of bound and free water, respectively. This equation assumes that the T_2 (and hence mobility) of all bound water molecules are equal. Thus, for total water contents below V_b, T_2^{-1} should be invariant with V, and for $V > V_b$, T_2^{-1} should be a linear function of V^{-1}, V_b being determined from the intersection of these two linear segments of a plot of T_2^{-1} vs. V^{-1}.

Figure 5 shows such a plot for ovalbumin and soy concentrate, consisting of two linear segments with intersections at 0.20 and 0.27 g of water/g of solids, respectively. Note that the $V \lesssim 0.3$ portion of the plot has a finite slope, rather than the zero slope predicted by the above equation. This suggests that although the data are consistent with the model assumed above (rapid exchange between two species of water with different mobilities), the bound water fraction is not homogeneous; there exists a range of water molecular mobilities within this species of water, with increasing average molecular mobility with increasing total water content. This situation is also supported by the spin–lattice (T_1) vs. temperature data (not reported), which show a broad minimum with $T_2 \ll T_1$ at the temperature of the T_1 minimum.

At water contents below about 0.07 g/g, the spin–spin relaxation times T_2 become too short to be accurately measured by our instrument. Thus, the water content region of the BET monolayer was not accessible for study by NMR. However, the fact that the relaxation behavior appears to change at about the BET monolayer water content suggests that there may be a change in the dependence of water molecular mobility on water content here, i.e., that the BET monolayer corresponds to tightly bound water (the least mobile species).

This hydration model, based on the NMR and BET results (tightly bound, bound, and free water), is consistent with the calculated thermodynamic parameters discussed above. The agreement between the total bound water contents determined by this NMR method and from the

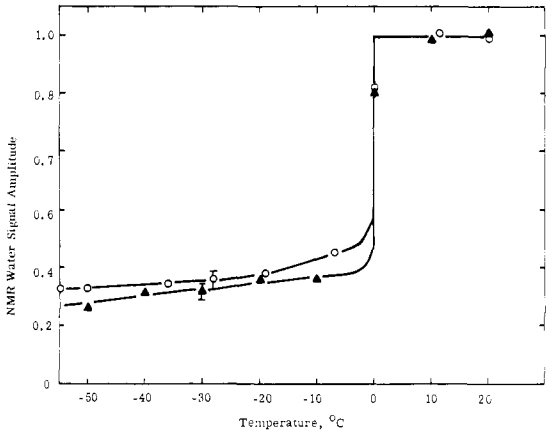

Figure 6. Water proton NMR signal amplitude (fraction of unfrozen water) vs. temperature for soy protein concentrate and ovalbumin. The standard deviation for each value, based on replicate measurements on the same sample, is indicated: (▲) soy protein concentrate; (○) ovalbumin.

Bradley isotherm treatment for soy concentrate and isolate is good, as shown in Table I, suggesting that the same water species is also being identified with these different techniques.

Measurement of the water NMR signal amplitude as a function of temperature is a second method of studying protein hydration, giving the fraction of water which does not freeze at temperatures below 0 °C, since the spectrometer used does not respond to ice NMR signals. Typical freezing diagrams for samples containing 1.0 g of water/g of soy concentrate and ovalbumin are shown in Figure 6. The signal amplitude is corrected as described earlier and normalized so that the amplitude at temperatures above 0 °C is 1.0. Between 0 and −10 °C, water begins to freeze. At −10 °C ~60% of the water has frozen. Thereafter only 10–15% more water freezes even down to −50 °C, so that the discrete fraction of unfrozen water is present. This water is presumably water of hydration of the protein and carbohydrate components which cannot adopt the ice structure due to interaction with these macromolecules. Knowing the fraction of total water unfrozen at −50 °C and the overall water content of the sample, the unfrozen water content is calculated to be 0.33 and 0.27 g of water/g of solids for ovalbumin and soy concentrate, respectively. These measurements were made at total water contents of 1 and 2 g of water/g of protein, to determine if the amount of unfrozen water was a function of total water content. These results are summarized in Table I. This second NMR method gives values of total bound (unfrozen at −50 °C) water in good agreement with the values obtained by the other methods reported here. In the case of ovalbumin, the unfrozen water value is slightly higher than the bound water values obtained by the other methods. This may be because the low-temperature NMR method is measuring not only water which does not freeze due to its interaction with protein (bound water), but also some water which cannot freeze due to being trapped in small spaces within the matrix of solid sample or of dissolved protein molecules. The value of 0.27 g of unfrozen water/g of solids obtained here for soy protein concentrate by NMR is remarkably close to that obtained by Okamura (1973) from freezing curves (area under temperature vs. time curves at different water contents) of whole soybeans. Okamura's value is 18.65% unfrozen water, which is 0.29 g of water per g of nonfat solids, based on an average oil content of 20% for his whole soybeans.

The results of these various methods for characterizing the state of water in soy protein are summarized in the following statements. (1) Water present up to ~0.065 g of water/g of solids is tightly bound (BET monolayer) and probably is water of hydration of ionic or highly polar protein (and carbohydrate, when present) 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. (4) The state of water in hydrated ovalbumin is very similar to that of soy protein concentrate, over the range of water contents studied here. Table I summarizes the data on water binding obtained by the various methods for the protein samples studied.

It is worth pointing out that these techniques cannot unambiguously distinguish between (1) complete coverage of exposed protein molecular "surfaces" with several adsorbed layers of water and (2) hydration of specific amino acid functional groups by particular numbers of water molecules characteristic of each amino acid. Surface areas measured for proteins by methods such as nitrogen sorption are probably not pertinent to water sorption, due to swelling of the hydrated protein with increasing water content. Kuntz et al. (1969) and Kuntz (1971a) have determined hydration numbers for the constituent amino acids of proteins, by measurement of the unfrozen water content of various polypeptides at −35 °C by a continuous-wave NMR technique. The bound (unfrozen at −35 °C) water contents of solutions of several proteins of animal origin and of known amino acid composition were then calculated, based on the amino acid hydration numbers. The agreement between these calculated values and those determined experimentally by NMR was generally quite good. The water-binding capacity of the protein portion of soy concentrate at pH 7 is ~0.35 g of water/g of protein, calculated using Kuntz (1971a) amino acid hydration numbers and the amino acid composition of the soy protein (Rackis et al., 1961). This value is somewhat higher than the ~0.25 g of water/g of solids obtained experimentally by our methods, suggesting that some of the potential water-binding sites in soy proteins are not exposed to water. The total water binding values observed for ovalbumin are also less than the 0.37 g/g hydration number calculated by Kuntz (1971b) for ovalbumin, similar to the situation found for soy protein.

Having a model for the equilibrium state of water in these proteins, both as a function of water content and activity, it is interesting to speculate on the relation between the state(s) of water and food stability. From data on the minimum water activities for nonenzymatic browning, enzyme activity, and growth of most bacteria in foods (Scott, 1957; Rockland, 1969; Bone, 1973; Labuza et al., 1970, 1972a,b; Mossel and Ingram, 1955; Plitman et al., 1973), it is possible to speculate on which of these states of water correspond to the occurrence of these various food degradation processes. Browning and enzyme activity both occur above the BET monolayer hydration value (tightly bound water), but well within the loosely bound water region. The NMR data show that the molecular reorientation rate ("mobility") of this monolayer water is quite low, compared to that of the other two water species; thus one might expect diffusion of reactants to be too slow for

enzyme or browning reactions to occur at appreciable rates, while the loosely bound water provides a matrix (or solvent) in which diffusion is rapid enough for these reactions to occur. The increase in browning and enzyme reaction rates with increasing water activity is similar to the measured increase in water molecular mobility which occurs in the loosely bound water region (see Figure 5). The water activity below which most bacteria will not grow (~0.9) corresponds to the transition between nonfreezeable (loosely bound) water and free water. It is tempting to speculate from this result that free water may be necessary to support bacterial growth. Because the water requirements of bacteria differ to some extent, it is clear that growth/no growth is dependent upon more than simply the presence of free water. In any case, it is clear that water activity, particularly in reduced moisture content foods, will be a function not only of the solute content, but also the nature of the food–water binding.

These correlations between the measured hydration state of the soy protein and rates of food degradation processes are speculative, since we do not have data on the latter for this material. The hydration model presented here does provide a sound basis for further experiments to investigate the reasons for the water activity/content dependence of degradation processes such as microbial growth, lipid oxidation, enzyme activity, and nonenzymatic browning, and to use hydration state as a means of food stability control.

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A Simple Method for Determination of Tryptophan in Food Samples

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A very simple and sensitive method for determination of tryptophan in food samples is described. The food proteins are completely solubilized by partial hydrolysis with papain in the presence of 8 M urea. An aliquot is diluted with 8 M urea and the fluorescence of tryptophan is directly measured at an emission wavelength of 348 nm, during excitation at 288 nm. Tryptophan is used as an internal standard. The results are compared with values obtained from a conventional method involving basic hydrolysis.

Tryptophan is one of the essential amino acids for human beings. Determination of the amount of tryptophan in foodstuffs is therefore of considerable importance. However, most of the methods used today are either laborious or not generally applicable. For a review on tryptophan analysis see Friedman and Finley (1975).

The native fluorescence of tryptophan is sufficiently intense and specific to permit its use for quantitative assay purposes (Udenfriend, 1962). The quenching effect of microenvironment on tryptophan fluorescence observed

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