Journal of Agricultural and Food Chemistry

Volume 26, Number 2 March/April 1978

Dehydration and Hydration Kinetics of Soybean Proteins

John R. Hansen

A model has been developed for the dehydration and hydration of soy proteins. The dehydration process for soy concentrate consists of an initial rapid step, presumably due to loss of surface and intercellular water, followed by a second slower step due to migration of water across the soybean cell walls. This final process is not only slower, but also has a higher apparent activation energy than the initial step. Disruption of the soybean cell structure by sonication or by extraction of cell wall material (e.g., as in isolation of protein from soy concentrate) results in loss of this final slow dehydration step. Dehydration rate constants and apparent activation energies are a function of the initial water content of the sample, with the former reaching a minimum constant value and the latter a maximum constant value (most unfavorable dehydration conditions) at ~1 g of water/g of solids. For vapor phase hydration of soy protein concentrate, random sorption of water on particle surfaces is followed by redistribution to the higher energy water-binding sites ("tightly bound" water) and next to lower energy sites ("loosely bound" water), followed by a final much slower movement of water across the soybean cell walls to intracellular sites.

Dehydration is one of the oldest known techniques for food preservation. The drying process has been extensively studied and modeled mathematically (Karel, 1975; Bagnoli et al., 1963; Van Arsdel et al., 1973; Desrosier, 1970). The literature on drying and rehydration of soybean protein materials is rather more sparse. Saravacos (1969) calculated the apparent diffusivity for sorption of water vapor by thin slices of soybeans (full-fat and defatted) and of freeze-dried whole soybeans. He found that the diffusivity was lower for full-fat slices than for defatted slices or freeze-dried whole beans and attributed this difference to the greater porosity of the latter two types of samples. Alam (1972) developed a model to simulate the deep-bed, low-temperature drying of soybeans, obtaining good agreement between calculated and experimental moisture gradients. The purpose of the present work was to gain some basic information about dehydration and rehydration kinetics of soybean proteins, in particular, how they depend on potential dehydration processing variables such as temperature and initial water content and on the form of the protein material (e.g., soy concentrate vs. isolate).

The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45247.

The approach taken here was to treat the dehydration/hydration rate data empirically and to gain knowledge of the system by relating changes in the kinetic parameters (e.g., rate constants) to changes in protein processing parameters (e.g., particle size) and dehydration/hydration conditions (e.g., temperature). The mathematical descriptions used for treatment of the data do not necessarily imply anything about the mechanisms of hydration and dehydration (e.g., diffusion vs. capillary flows) and were chosen because of their utility in describing the dehydration/hydration behavior of human stratum corneum (Anderson et al., 1973).

EXPERIMENTAL SECTION

Dehydration experiments were carried out on a DuPont Model 951 thermogravimetric analyser (TGA) attached to a DuPont 900 differential thermal analysis unit. The TGA unit is an electrobalance whose sample arm can be enclosed in a furnace, the temperature of which can be varied between room temperature and 500 °C and through which dry nitrogen is passed. Sample weight is monitored as a function of time at a known temperature and then converted to water content vs. time from the known initial water content. The sample temperature measurement thermocouple was placed within ≤1 mm of the sample and

0021-8561/78/1426-0297$01.00/0

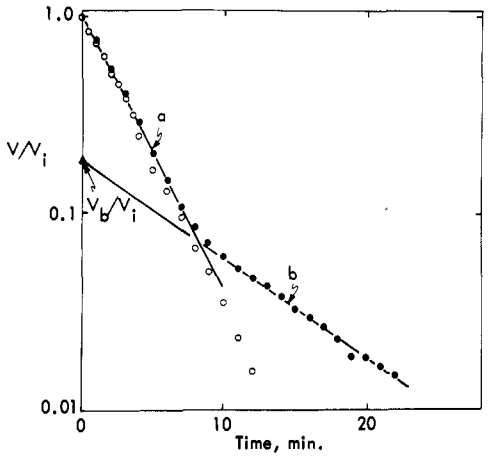

Figure 1. Dehydration of soy protein concentrate: V = water content (g of water/g of solids) at time t; V_i = initial water content at time zero. Initial and final processes are indicated by a and b, respectively, as defined in the text. (●) 2.0 g of water/g of solids initially, 125 °C; (○) 2.0 g of water/g of solids initially, sonicated, 120 °C.

thus measures the temperature of the gas passing over the sample. Typical sample weights were 100 mg, measured with a precision of ±0.1 mg. The major source of error in these measurements is control of sample temperature, particularly at the beginning of the experiment when a sample of known weight is placed in the balance pan and enclosed in the preheated furnace, which cools down to some extent due to being opened. Hydration (vapor phase) experiments were carried out using a Cahn RG electrobalance equipped with a glass chamber through which air containing water at various activities was circulated. This air of known a_w was obtained by passing air through a sintered glass filter immersed in a saturated salt solution of known a_w (Young, 1967). Sonication of soy protein concentrate was carried out as previously described (Hansen, 1977).

Soy protein concentrate, prepared as previously described (Hansen, 1976), and Anderson Clayton Foods ACP-950 soy protein isolate were used. The soy concentrate is 69% protein (% N × 6.25), 0.4% lipid (petroleum ether extract), and 6.1% ash, while the corresponding values for the soy isolate are 95% protein, 0.1% lipid, and 4.5% ash. Different particle size ranges of soy concentrate were obtained by shaking soy concentrate through screens of various mesh sizes.

RESULTS AND DISCUSSION

Dehydration. Figure 1 shows the loss of water from soy protein concentrate as a function of time at 125 °C for a sample initially containing 2 g of water/g of solids. This water content vs. time data can be fit by two first-order (with respect to time) processes, indicated by a and b in the figure. In this treatment of the data, no significance in terms of the water loss mechanism is claimed. It is simply a convenient way of comparing data on different samples as parameters such as temperature and relative humidity of the drying environment are varied. The dehydration process is thus treated as consisting of two first-order processes, $dV/dt = K^1V$, each with a rate constant K^1_a and K^1_b, for the initial and final steps, respectively, and amounts of water V_a and V_b associated, respectively, with these two steps (V is the water content at any time t, and V_i is the initial (t = 0) water content in g of water/g of solids). As shown in Figure 1, V_b is obtained by extrapolation of the long-time portion of a plot of ln (V/V_i) vs. time, V_a is $(1-V_b)V_i$, and K^1 are the slopes

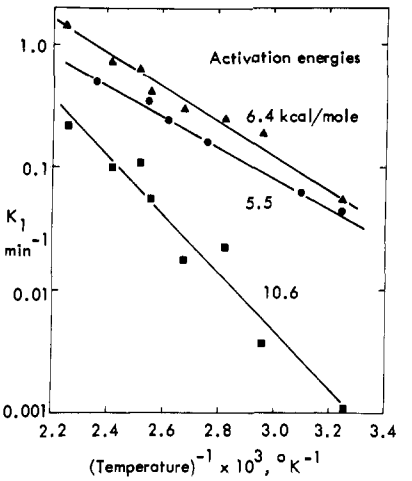

Figure 2. Arrhenius plots of dehydration rate constants for 2.0 g of water/g of soy protein concentrate: (▲) soy concentrate, K_1^a (initial fraction of water lost); (■) soy concentrate, K_1^b (final fraction of water lost); (●) sonicated soy concentrate.

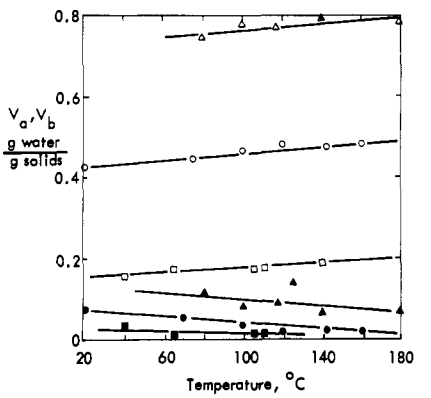

Figure 3. Amounts of water associated with the initial and final dehydration processes for soy protein concentrate, at various temperatures and initial water contents: (□, V_a; ■, V_b) $V_{initial}$ = 0.19 g/g; (○, V_a; ●, V_b) $V_{initial}$ = 0.50 g/g; (△, V_a; ▲, V_b) $V_{initial}$ = 0.86 g/g.

of the portions a and b of this plot.

In order to determine apparent activation energies for the dehydration processes, rate constants were measured at several temperatures for samples of several initial water contents. The data for a sample containing 2 g of water/g of solids initially are shown in Figure 2 as an Arrhenius plot of ln K^1 vs. $1/T$. It is clear that not only is the rate constant for the final dehydration step smaller than for the initial step, but also the activation energy is greater. The activation energy for the initial process is only ~2 kcal/mol higher than that for the diffusion of water in water (Hindman, 1974), while that for the final process is more like the activation energy for diffusion of water through solid polymeric membranes (Barrie and Machin, 1971).

The amounts of water associated with each dehydration process, as determined from plots like that in Figure 1, are shown in Figure 3 as a function of temperature for samples of three different initial water contents. The variation in relative amounts of the "types" of water, V_a and V_b, with temperature is not large. In addition, the ratio of amounts, V_a/V_b, is almost constant for different initial water contents. For example, at 20 °C, V_a/V_b is 5.0, 6.2, and 5.8 for initial water contents of 0.19, 0.50, and 0.86 g of water/g of solids, respectively. This result shows that the water being lost by these two processes does not correspond to "free" and "bound" water, as the ratio of free to bound

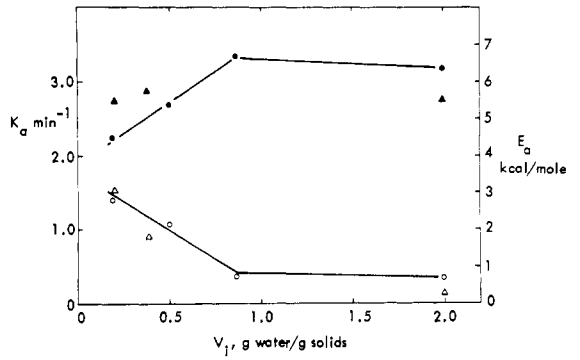

Figure 4. Dehydration rate constants and activation energies for soy protein concentrate and isolate at 100 °C as a function of the initial water content, V_i: (○, K_a; ●, E_a) soy protein concentrate; (△, K_a; ▲, E_a) ACP-950 soy isolate.

water for samples of these three water contents is 0, 1.0, and 2.4, respectively (Hansen, 1976).

The difference in activation energies for the two dehydration processes and the lack of correlation of the amounts of water associated with each process with equilibrium water "binding" suggests that the two dehydration steps represent loss of water from two different locations in soy concentrate, one of which has a higher energy barrier to water loss. A reasonable postulate is that the initial faster step is due to loss of surface and intercellular water, while the final step (lower rate constant and higher activation energy) is due to water diffusing through the soybean cell wall. This view is supported by the data in Figure 1, which shows that for soy concentrate whose cell structure has been completely disrupted by ultrasonic irradiation, only the initial rapid dehydration process remains—the second slower step is missing. Also, as shown in Figure 2, the activation energy for dehydration of sonicated soy concentrate is similar to that for the rapid initial dehydration step in intact soy concentrate.

Figure 4 shows the dependence of dehydration rate constants and activation energies on initial (prior to dehydration) water content for soy protein concentrate and isolate. For soy concentrate, when plotted against initial water content, the activation energy is seen to increase up to ~1 g of water/g of solids and then levels off. Likewise, the rate constant for dehydration decreases up to ~1 g of water/g of solids and then levels off. These results indicate that the rate and energetics of the dehydration process for soy concentrate are least favorable at initial water contents above ~1 g of water/g of solids and become more favorable at lower initial water contents. This result suggests that the barrier properties of the water-loss barrier are a function of water content, similar to the situation found for dehydration of human stratum corneum (Anderson et al., 1973).

The effect of protein particle size prior to hydration on dehydration kinetics was also examined. Double exponential dehydration curves similar to that shown on Figure 1 were obtained for soy protein concentrate which had been screened to give particle size ranges of 44–74 and 350–420 μm. Arrhenius plots of the initial rate constant vs. T^{-1} for these two samples are similar to those shown in Figure 2 for whole soy concentrate, but not identical. The apparent activation energies for the large and small particle size samples are 5.6 ± 0.9 and 2.9 ± 0.7 kcal/mol, respectively. At first glance, this result seems surprising (larger surface area/g of samples have higher activation energy). However, the results are consistent with the dehydration model developed. This model states that intact soybean cell walls are a major barrier to water loss,

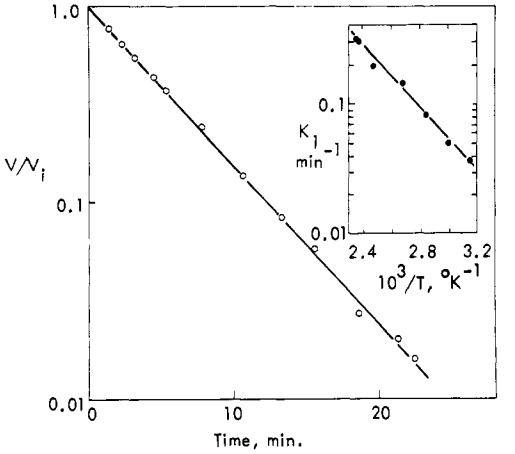

Figure 5. Dehydration of soy protein isolate ACP-950: V = water content (g of water/g of solids) at time t; V_i = initial water content at time zero = 2.0; temperature 130 °C (○) and Arrhenius plot of dehydration rate constant K_1 vs. T^{-1} (●).

Table I. Dehydration Rate Constants at 100 °C (K_1) and Apparent Activation Energies (E_a) for ACP-950 Soy Protein Isolate at Different Initial Water Contents (V_i)

V_i, g of water/g of solids	K_1, min^{-1}	E_a, kcal/mol
2.0	0.14	5.5
0.38	0.90	5.8
0.20	1.5	5.5

while loss of water from the microscopic surface of the object being dried occurs more rapidly and with a lower apparent activation energy. When the 44–74 and 350–420 μm particles are hydrated, they form a coherent mass, whose effective surface area for water loss is determined by the size of the mass, not of the individual particles which went into its making, and which still contain the microscopic barriers to water loss—the soybean cells. Thus, particle size prior to hydration has only a small effect (compared to the experimental errors) on dehydration kinetics. The difference observed is probably due to the lower fraction of intact cells in the small particle size material.

Figure 5 shows the dehydration curve for soy protein isolate ACP-950. Dehydration occurs by means of a single first-order (with respect to time) process, with rate constants similar to those found for the initial dehydration process in soy concentrate. These rate constants were measured at several temperatures and are plotted vs. $1/T$ on the inset of Figure 5. The apparent activation energy thus obtained for the dehydration process is 5.5 kcal/mol. These findings support the hypothesis that the final dehydration process observed in soy concentrate is due to transfer of water across the cell membrane. The soybean cell structure is completely disrupted in soy isolate, and the rate constants and activation energy for dehydration of soy isolate are very similar to those for the initial dehydration process in soy concentrate. Soy protein isolate shows a dependence of dehydration rate constants on initial water content similar to that shown by soy concentrate, but not for activation energies (Figure 4). Table I shows rate constants and activation energies for ACP-950 soy isolate at three initial water contents: the rate constant decreases with increasing initial water content and the activation energy is constant. The lack of dependence of activation energy on initial water content in the case of soy protein isolate is surprising, perhaps suggesting that the dependence of the dehydration kinetic parameters on

Table II. Rate Constants and Amounts of Water Associated with the Three Processes in the Vapor Phase Hydration of Soy Concentrate at Different Water Activities (a_w) at 21 °C

	Amounts of water (g of water/g of solids)			Rate constants $\frac{\text{g of water}}{\text{g of solids min}}$ for K_0; min^{-1} for K_1' and K_1		
a_w	V_1' (initial process)	V_1 (2nd)	V_0 (final)	K_1' (initial process)	K_1 (2nd)	$K_0 \times 10^4$ (final)
0.79	0.029	0.113	0.009	0.18	0.033	0.18
0.95	0.041	0.18	0.049	0.11	0.03	1.3
0.98	0.068	0.21	0.063	0.21	0.02	1.7
Sample below brought to equilibrium at 0.19 g of water/g of solids (a_w 0.79), then hydrated at a_w 0.98.						
0.98	0.018	0.05	0.06	0.05	0.007	0.3

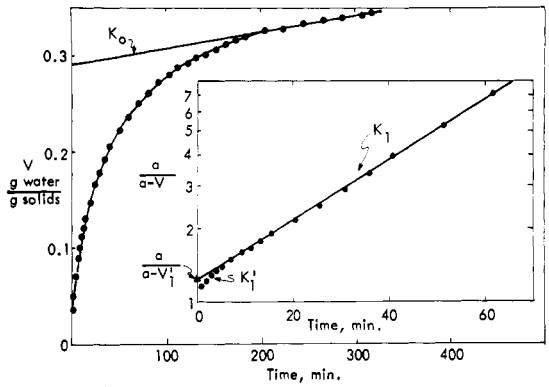

Figure 6. Hydration of soy protein concentrate from dryness in a 0.98 a_w environment. The inset is a first-order (with respect to time) plot of the short-time data.

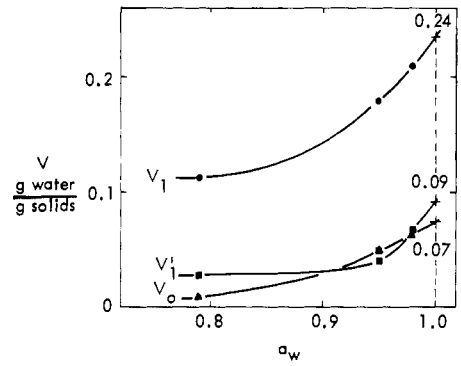

Figure 7. Amounts of water associated with each kinetic hydration process at different hydration water activities, V_1', V_1, and V_0 are the amounts of water associated with the initial and second first-order and final zero-order hydration processes, respectively. The numbers on the right side of the figure are the values extrapolated to unit a_w.

initial water content in the case of soy protein concentrate is attributable to the cell wall structure, which has maximum resistance to passage of water above ~1 g/g of solids.

Hydration. Figure 6 shows the weight of water gained per unit weight of dry soy protein concentrate as a function of time from vapor phase hydration at 21 °C in an environment of 0.98 a_w. Most of the water sorption takes place via two processes: (a) an initial phase that is approximately first order (with respect to time), described by: $dV/dt = K_1(a - V)$, $0 < V < a$; (b) a slower zero order phase described by: $dV/dt = K_0$, $V > a$. Here V is the weight of water sorbed per unit dry tissue weight at time t, K_1 and K_0 are the first- and zero-order sorption rate constants, respectively, and a is the weight of water sorbed by the first-order process.

The rate constant K_0 is determined from the slope of the final linear portion ($V > a$) of the V vs. t plot. The value of a is found by extrapolation of this portion of the curve to zero time as shown in Figure 6. That most of the initial sorption is approximately first order can be seen from the linearity (except at short t) of a plot of ln (a/(a–V)) vs. time, shown in the inset of Figure 6. The initial nonlinearity of this curve suggests that the initial first-order process is actually two processes. If we let $a = V_1 + V_1'$, where V_1' is the value of V when ln (a/(a – V)) is extrapolated to zero time (Figure 6), and K_1' be the slope of a plot of the difference between ln $(a/(a - V))$ and $K_1't + a/(a - V_1')$, then the initial sorption phase can be treated in terms of two pseudo-first-order processes, with rate constants K_1' and K_1, and amounts of water sorbed by each V_1' and V_1. These two processes are followed by the final zero-order phase with rate constant K_0 and amount sorbed V.

This hydration experiment was carried out for soy concentrate at three hydrating a_w's: 0.79, 0.95, and 0.98. The rate constants and amounts of water sorbed by means of each process are shown in Table II. The amounts of water sorbed by each process increase with increasing hydration a_w, as does the equilibrium water content. The rate constant K_0 for the final slow hydration process also increases greatly with increasing hydration a_w. However, the rate constants for the initial hydration processes appear to be invarient with a_w. These results suggest that the barrier to water sorption corresponding to the slow final hydration process is a strong function of water content, while that of the initial processes is not. A reasonable explanation is that the initial processes correspond to random surface water sorption and redistribution to higher energy surface and intercellular water sorption sites, while the final process is migration of water across the soybean cell walls to the intracellular protein. This model is similar to the dehydration model.

If the amounts of water associated with each process are plotted vs. a_w (Figure 7) and extrapolated to $a_w = 1$ (saturated water vapor), the amounts of water sorbed by the initial first-order processes, V_1' and V_1, are quite similar to the equilibrium "tightly bound" and "loosely bound" water values obtained for soy protein concentrate (Hansen, 1976). This may be coincidental, but supports the above model in which these initial processes represent random surface water sorption followed by redistribution first to the highest energy (tightly bound) sites and then to the so-called loosely bound sites.

Table II also shows data for the hydration at $a_w = 0.98$ of soy concentrate which had previously been equilibrated at $a_w = 0.79$ to 0.19 g of water/g of solids. The amounts of water sorbed by the initial first-order processes are much smaller than those for materials hydrated from dryness, consistent with the postulated model, since most of the "bound" water sites are already occupied in this preequilibrated sample. Somewhat surprising is the slowness of the hydration process in this sample—the rate constants being ca. three–five times smaller than for the sample

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.

LITERATURE CITED

Alam, A., Ph.D. Dissertation, University of Illinois, 1972.
Anderson, R. L., Cassidy, J. M., Hansen, J. R., Yellin, W., *Biopolymers* **12**, 2789 (1973).
Bagnoli, E., Fuller, F. H., Norris, R. W., in "Chemical Engineers Handbook", 4th ed, Perry, J. H., Ed., McGraw-Hill, New York, N.Y., 1963, Section 15.
Barrie, J. A., Machin, D., *Trans. Faraday Soc.* **67**, 244 (1971).
Desrosier, N. W., "The Technology of Food Preservation", 3rd ed, Avi Publishing Co., Westport, Conn., 1970, Chapter 5.
Hansen, J. R., *J. Agric Food Chem.* **24**, 1136 (1976).
Hansen, J. R., submitted to *J. Agric. Food Chem.* (1977).
Hindman, J. C., *J. Chem. Phys.* **60**, 4488 (1974).
Karel, M., "Principles of Food Science", Part II, Fennema, O. R., Ed. Karel, M., Fennema, O. R., Lund, D. B., Ed., New York, N.Y., 1975, Chapters 7, 9–11.
Saravacos, G. D., *Food Technol.* **23**, 1477 (1969).
Van Arsdel, W. B., Copley, M. J., Morgan, A. I., Jr., Ed., "Food Dehydration", 2nd ed, Vol. I, Avi Publishing Co., Westport, Conn., 1973.
Young, J. F., *J. Appl. Chem.* **17**, 241 (1967).

Received for review July 28, 1977. Accepted November 22, 1977.

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

John R. Hansen

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.

The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45247.

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

0021-8561/78/1426-0301$01.00/0