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Desorption and Adsorption Isotherms of Meat–Salt Mixtures

Theodore S. Lioutas, Peter J. Bechtel, and Marvin P. Steinberg*

Water binding by muscle proteins in the presence of different levels of salt was studied. Biceps femoris muscle was ground and dialyzed against distilled water. Increasing levels of salt were added to seven aliquots. Different levels of salt were added to each of seven aliquots of ground muscle. Half of each aliquot was taken for desorption studies; the other half was freeze-dried for adsorption studies. Isotherm data were obtained by equilibration to saturated standard salt solutions. Samples containing no salt had similar adsorption and desorption equilibrium moisture values and salt alone showed no sorption below $0.75a_w$ and a large hysteresis effect above $0.75a_w$. Increasing the salt content dramatically increased the moisture content at comparable a_w above 0.75, and the salt–muscle protein mixtures showed hysteresis.

Water and protein interactions have been intensively investigated over the last 10 years (Karel, 1973; Kuntz and Kauzmann, 1974). Early studies on the sorption of water vapor by proteins (Sponsler et al., 1940; Shaw, 1944; Bull, 1944; Pauling, 1945) found that the amount of water adsorbed depended on the number and availability of the two types of hydrophilic groups binding water molecules through hydrogen bonding. Cassie (1945) and Pauling (1945) showed that water vapor was adsorbed by these hydrophilic groups at lower water activities (a_w) and that multimolecular water adsorption occurs at higher a_w.

It has been found that the amount of water adsorbed by proteins at an a_w of 0.5 or below fits the Langmuir adsorption isotherm (Bull, 1944) and that the multimolecular adsorption of water at these a_w levels can be predicted by the BET equation (Brunauer et al., 1938; Shaw, 1944; Pauling, 1945; Guggenheim, 1966; Rochester and Westerman, 1976). However, Iglesias and Chirife (1976) and Labuza (1975) found that the BET equation does not accurately estimate the moisture conent at high a_w values (greater than 0.5) such as those associated with the physical and chemical deterioration of foods. Analysis of the system becomes more complicated with the addition of other components such as salt.

One of the many effects of sodium chloride in food systems is that it decreases a_w (Ingram and Kitchell, 1967). Most processed meat products contain sodium chloride, which adds sensory, functional, and preservation properties to the products. The trend toward development of more intermediate moisture meat products has placed a greater emphasis on understanding the equilibrium moisture characteristics of the meat proteins in conjunction with NaCl. The physical and chemical properties of many proteins are affected by ionic strength (Arakawa and Timasheff, 1982); thus, salt content affects the water binding properties of the proteins, which in turn affect food preservation. Previous studies have found that the water binding properties of proteins such as lysozyme (Hnojewyj and Reyerson, 1961), soy protein (McCune, 1981) and casein (Hardy and Steinberg, 1984) vary with the sample preparation and whether the equilibration was done from the wet or dry state.

The objective of this study was to obtain sorption isotherms for meat proteins containing different levels of salt in order to increase our knowledge of the effect of salt on meat systems under both desorption and adsorption conditions.

MATERIALS AND METHODS

Preparation of Samples. Bovine biceps femoris muscle was removed 8 h after slaughter, and excess fat and connective tissue were removed (Figure 1). The muscle was ground through a 3 mm hole plate and blended for 10 s in a Waring blender with 2 volumes of cold water. The homogenate was exhaustively dialyzed against three changes of cold water for a total of 3 days to remove inorganic salts and small molecular weight compounds. The dialysate contained 2 mM sodium azide to inhibit bacterial growth. The dialyzed sample was then split; one aliquot was freeze-dried and served as the dry control (sample 8, Table I), and the other aliquot was concentrated under vacuum at 4 °C over $CaCl_2$ in order to remove excess water and thus shorten equilibration time. An aliquot of this concentrate was removed for the wet control (sample 0, Table I). The concentrate was then divided into seven parts, and salt was added at different levels (samples 1–7, Table I). After salt addition, the samples were stored at 20 °C for 6 h; half of each sample was utilized for the desorption study, and the other half was freeze-dried for the adsorption study.

Isotherms. The isotherm data were obtained by using the proximity equilibration cell described by Lang et al. (1981) modified as specified below. All measurements were obtained at 5 ± 1 °C, and the different saturated salt solutions and all a_w values, some of which were taken from the literature and some of which were measured in this study, are given in Table II. All desorption and adsorption

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Table I. Composition of Meat and Salt Mixtures Used for Adsorption and Desorption Experiments[a]

sample no.	water, % (W)	solids total	protein, % (P)	NaCl, % (S)	other[c] solids, %	S/P	W/S
0	88.05	11.95	9.66	0.09[b]	2.29	0	0
1	86.37	13.63	10.58	0.54	2.51	0.0515	159.94
2	85.93	14.07	10.53	1.05	2.49	0.1000	81.84
3	85.03	14.97	10.42	2.08	2.47	0.2000	40.88
4	83.30	16.70	10.21	4.08	2.41	0.4000	20.42
5	83.23	16.77	9.13	5.48	2.16	0.6000	15.19
6	79.25	20.75	9.71	8.74	2.30	0.9000	9.07
7	76.90	23.10	8.44	12.66	2.00	1.5000	6.07
8	5.84	94.16	76.04	0.68[b]	17.44		
9	0.20	99.80	0	99.80	0		
10	90.00	10.00	0	10.00	0		

[a] Values expressed on a percent wet weight basis. [b] Values express the total KCl and NaCl content on a total weight basis calculated from atomic adsorption analysis. [c] Other solids are fat and inorganic constituents.

Table II. Water Activity of the Saturated Salt Solutions at 5 °C

salt	a_w at 5 °C	salt	a_w at 5 °C
K_2SO_4[a]	0.9848	$NaNO_3$[a]	0.7857
KNO_3[a]	0.9627	NaCl[b]	0.7505
$ZnSO_4$[b]	0.9215	NaBr[a]	0.6351
$BaCl_2$[b]	0.9135	K_2CO_3[a]	0.4313
KCl[a]	0.8767	LiCl[a]	0.1126
$(NH_4)_2SO_4$[b]	0.8242		

[a] Values taken from Greenspan (1977). [b] Values were determined experimentally with the isopiestic method (McCune et al., 1981) using as standards the values from Greenspan (1977).

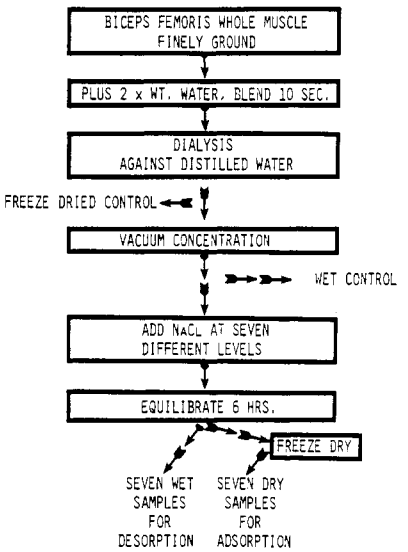

Figure 1. Sample preparation procedures for isotherm studies.

mixtures were equilibrated in duplicate over the saturated salt solutions for 15 days. Previous studies have shown that 15 days was sufficient time for equilibration. The sodium chloride desorption and adsorption samples (10 and 9, Table I) were equilibrated in triplicate for 30 days because of the large amount of water involved.

For adsorption, 1 g of dry mixture was placed on plastic gauze instead of filter paper to prevent possible interaction of the salt with the paper. For desorption, 3 g of wet mixture was placed in a tared cup held in the bottom of the cell such that the surfaces of the saturated salt and sample were at the same level. The latter arrangement was also used for both adsorption and desorption of salt alone.

Moisture content was determined by weight loss after drying the samples at 25 mmHg pressure and 60 °C for 24 h (AOAC, 1981). Protein was analyzed by the Kjeldahl method (AOAC, 1981), and the protein content was calculated by using the constant 6.25 to convert Kjeldahl nitrogen to protein. The sodium chloride and potassium

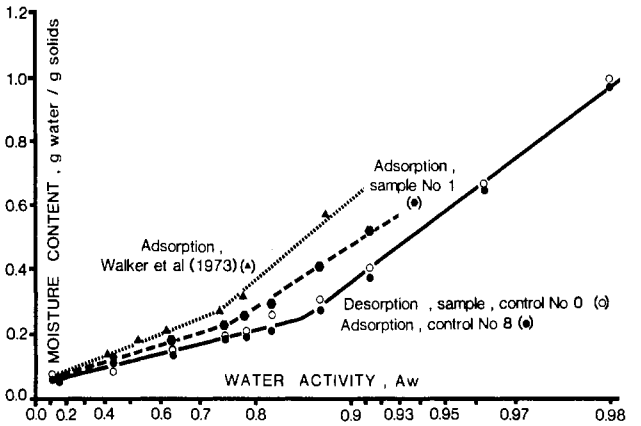

Figure 2. Adsorption and desorption of dialyzed meat controls as compared to adsorption of dialyzed meat containing added salt at the level found in muscle and to literature values for meat (undialyzed).

chloride content of the dialyzed freeze-dried control (sample 8, Table I) was determined to be 0.68% (wet basis) by atomic adsorption analysis of sodium and potassium content. This value was used to calculate the sodium chloride and potassium chloride content of the wet control sample (sample 0, Table I) by assuming the percentage of salt in the solids was constant.

RESULTS AND DISCUSSION

Meat Protein Isotherms. The moisture sorption characteristics of raw freeze-dried beef (Saravacos and Stinchfield, 1965; MacKenzie and Luyet, 1967), precooked freeze-dried beef (Kapsalis et al., 1964), and raw muscle and mosyin extracts (Walker et al., 1973) have been reported. In this study designed to examine the effect of salt (NaCl) on the sorption characteristics of skeletal muscle proteins, the ground muscle samples had to be dialyzed exhaustively against water to remove NaCl and KCl intrinsic to skeletal muscle. Both adsorption and desorption isotherms were determined at 5 °C for the dialyzed whole muscle homogenates. The results of the experiments are plotted according to Smith (1947) and Lang and Steinberg (1981) as linear isotherms (Figure 2). The data points for the desorption and adsorption isotherms were similar so a single curve was constructed. In this study, the dialyzed meat showed no hysteresis; however, Walker et al. (1973) used myosin isolates at different temperatures and noted that the isotherms displayed a slight hysteresis component. The curves for the control samples in Figure 2 were composed of two straight lines that intersect at about $0.86a_w$ for both adsorption and desorption and indicated a change in the water binding characteristics.

The dialysis step needed in these experiments to remove salt intrinsic to skeletal muscle could have resulted in

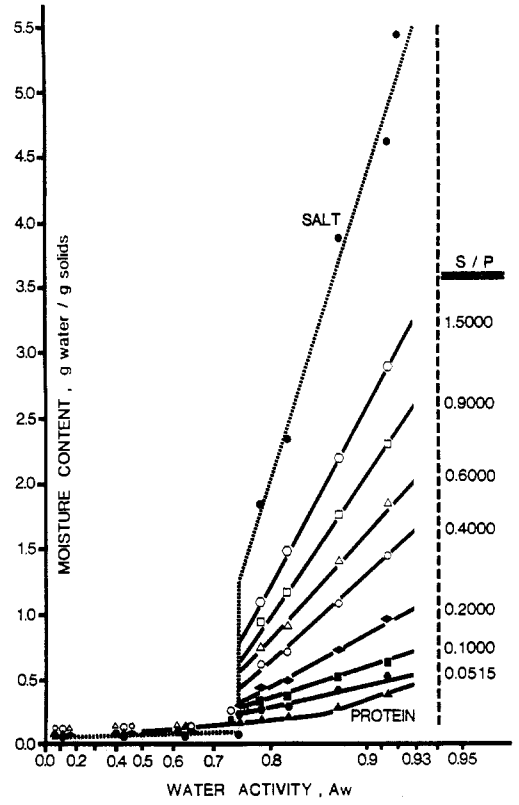

Figure 3. Adsorption isotherms at 5 °C for sodium chloride, freeze-dried dialyzed protein, and mixtures of the two. S/P is the weight ratio of salt to protein. The solid circles with slashed line are for salt, open hexagons are S/P of 1.500, open squares are S/P of 0.900, open triangles are S/P of 0.600, open circles are S/P of 0.400, solid diamonds are S/P of 0.200, solid squares are S/P of 0.020, solid hexagons are S/P of 0.0515, and solid triangles are protein.

swelling or other disruptions of the myofibrillar lattice. However, from Figure 2, the adsorption isotherm data for whole muscle reported by Walker et al. (1973) is in general agreement with the adsorption data from this study for dialyzed meat to which a small amount of salt has been added (sample 1, Table I).

Salt Isotherms. The saturation a_w of NaCl was determined to be 0.7505 by using the proximity equilibration cell with the isopiestic technique developed by McCune et al. (1981). The adsorption and desorption for sodium chloride isotherms at 5 °C are presented in Figure 3 and 4. Salt bound a very small amount of water at an a_w below 0.75, and above this a_w there was a large water sorption that increased rapidly with small increases in a_w.

Data points were obtained up to $0.92a_w$ for both adsorption and desorption. In the desorption case, five data points were obtained between an a_w of 0.75 and 0.92 similar to the adsorption case (Figure 3). However, only two points were displayed for the salt curve in Figure 4 for graphical reasons. These omitted points (first the a_w value and then the corresponding moisture content in grams of water per 100 g of solids) were as follows: 0.8767, 7.02; 0.9135, 9.34; 0.9215, 10.57. The slopes and intercepts (Smith parameters) of the linear regression lines were calculated and are listed in Table III along with the linear log correlation coefficient for all samples. The vertical line drawn for sodium chloride at $0.75a_w$ indicates the measured saturation a_w at 5 °C using the isopiestic method (McCune et al., 1981). The salt curve in Figure 4 shows the desorption data for sodium chloride in which the moisture content for a saturated sodium chloride solution at 5 °C was taken from Weast and Astle (1982). This handbook

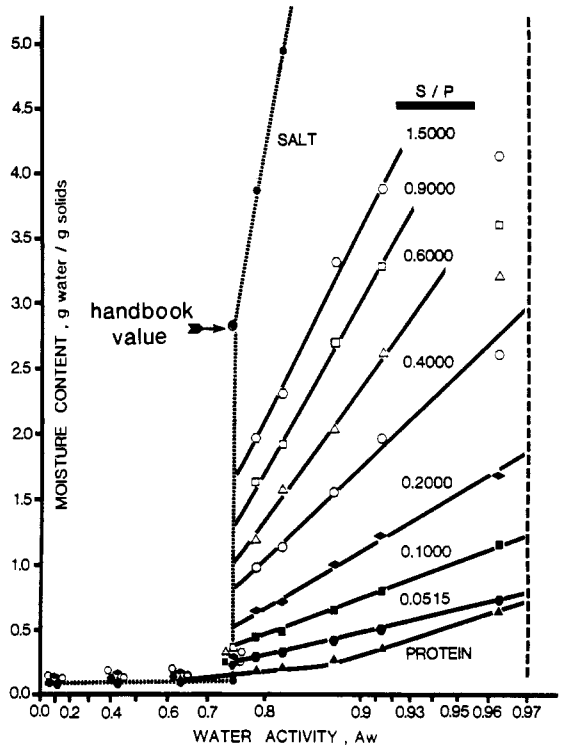

Figure 4. Desorption isotherms at 5 °C for sodium chloride, dialyzed meat proteins, and mixtures of the two. Symbols are the same as in Figure 3.

Table III. Calculated Parameters for Adsorption and Desorption Samples[a]

sample no.	a (slope)	b (intercept)[b]	r (linear log correlation coefficient)
desorption			
0	−0.4278	−0.0647	0.9930
1	−0.5564	−0.0749	0.9982
2	−0.9356	−0.1929	0.9987
3	−1.3785	−0.2869	0.9987
4	−2.4537	−0.6684	0.9981
5	−3.4183	−1.0359	0.9989
6	−4.4970	−1.4246	0.9995
7	−5.0734	−1.4376	0.9961
10	−14.9370	−6.3006	0.9955
adsorption			
1	−0.6883	−0.1925	0.9921
2	−0.8189	−0.2549	0.9939
3	−1.3451	−0.4958	0.9945
4	−2.1546	−0.8712	0.9963
5	−2.8458	−1.1940	0.9981
6	−3.6045	−1.5418	0.9978
7	−4.5931	−1.9954	0.9999
8	−0.3556	−0.0341	0.9687
9	−7.9216	−3.5212	0.9901

[a] Values calculated by the method of Smith (1947). [b] Intercept of y axis is zero a_w.

moisture content was plotted on the vertical line at the determined a_w of saturation and falls in the linear part of the isotherm drawn from five experimental points between an a_w of 0.75 and an a_w of 0.92. The desorption salt isotherm indicated a higher moisture content at comparable a_w than adsorption isotherms; similar salt hysteresis was observed by Sloan and Labuza (1975), who explained it as a supersaturation effect.

Protein–Salt Isotherm Data. The adsorption and desorption isotherms for mixtures of salt and protein (1–7, Table I) are shown in Figures 3 and 4. The range over which the Smith plots gave linear adsorption isotherms was an a_w of 0.75–0.93 and the desorption isotherms

0.75–0.965 for S/P values ranging from 0.0515 to 0.400.

All protein–salt mixtures (samples 1–7, Table I) have plots similar in shape to that of salt; they adsorbed water above an a_w of 0.75 and little moisture below that point. Figures 3 and 4 indicate that as the salt content increased, the slope of the lines increased above an a_w of 0.75 with increasing salt content (Table III). The isotherms approached that of pure salt as the salt content increased. Thus, salt content was the most important factor controlling the equilibrium moisture in this study.

The present technique of starting with dialyzed muscle proteins and adding known amounts of salt before equilibration to known a_w has enabled us to quantitate the effect of salt on water binding in both desorption and adsorption modes. Although the dialyzed protein isotherms did not show a hysteresis effect, the isotherms of the salt–protein mixtures showed strong hysteresis effects with the desorption isotherms having the higher moisture contents. The effect of hysteresis became larger with increasing salt content. This suggests that the hysteresis effect observed for the salt–protein mixtures was due to the salt, which showed a large hysteresis effect by itself.

An interesting application of these data is to assume that a processed meat contains 3% total salt and 20% protein (S/P = 0.15) and that preservation is assured at $0.85a_w$. Figure 4 shows that meat can contain as much as 1.1 g of water/g of solids with this salt content and be shelf stable. Thus, the above addition of salt to the processed meat with an additional adjustment of the moisture content enabled preservation while retaining almost 3 times as much water.

Registry No. NaCl, 7647-14-5; $ZnSO_4$, 7733-02-0; $BaCl_2$, 10361-37-2; $(NH_4)_2SO_4$, 7783-20-2; water, 7732-18-5.

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Effects of Drying on Selected Qualities of *Spirulina platensis* Protein

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Spirulina platensis was collected after culture and dried in four ways: freeze, drum, cabinet, and solar. Available protein lysine was increased in the treatments involving drying at elevated temperatures, and in vitro enzymatic protein digestibility was increased in all samples over that of an undried control.

The production of alternate sources of protein for human and animal needs has become increasingly important. *Spirulina platensis* is a blue-green algae (Cyanobacterium) that has been found to exceed 50% crude protein (Anusuya Devi et al., 1981). A closely related species, which may actually be synonymous with *S. platensis*, is *Spirulina maxima*, which has been found to contain from 60 to 71% crude protein. This is a high-quality protein, with a digestibility of 76–84%, a protein efficiency ratio (PER) of 2.2–2.6 (74–87% that of casein), and a biological value (BV) of 60–72 (Clement et al., 1967; Skowronski, 1980). The use of *Spirulina* as a high-protein feed supplement has recently been identified by the State of Hawaii as a research priority (Anonymous, 1982).

After collection of the *Spirulina*, the raw product is approximately 90% moisture, which presents a storage problem in terms of both bulk and perishability. These two problems are easily solved by drying. However, drying can induce changes in the quality of the protein (Wolf et al., 1978; Adrian 1975). This study was undertaken to

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