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MOISTURE ADSORPTION BY GRAIN PROTEIN FILMS

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ABSTRACT. Grain protein films plasticized with glycerol were prepared from corn zein (CZ), wheat gluten (WG), and a 2.3:1 mixture of wheat gluten and soy protein isolate (WG/SPI). Moisture adsorption curves of the three types of protein films at 25° C and within an approximate water activity range of 0.11 to 0.84 were obtained using a static gravimetric method. The Smith, Oswin, Halsey, and Guggenheim-Anderson-de Boer (GAB) models were applied to collected data. For all protein films, the GAB model showed the best fit over the entire studied water activity range yielding mean deviation modulus values (*P*) of 4.69, 3.44, and 7.95 for WG, WG/SPI, and CZ films, respectively. The Smith and Halsey models fitted well the high water activity (0.53 to 0.84) portion of the isotherms with *P* values ranging between 2.55 and 6.74. Moisture adsorption behavior by the protein films at the low water activity range (0.11 to 0.58) was reasonably well described by the Oswin model (*P* values of 6.68, 5.25, and 5.24 for WG, WG/SPI, and CZ films, respectively).

Keywords. Protein films, Moisture adsorption, Corn zein, Wheat gluten, Soy protein.

Edible biopolymer films and coatings derived from protein, polysaccharide, and lipid materials have been investigated for potential applications in food packaging and food preservation. Edible film formation mechanisms, production methods, and properties have been comprehensively reviewed (Guilbert, 1986, 1988; Kester and Fennema, 1986; Krochta, 1992). Detailed discussions on protein films in particular have also been published (Gennadios and Weller, 1990, 1991; Gennadios et al., 1993a). Several recent studies have reported information on mechanical and barrier properties of protein films from wheat gluten (Park and Chinnan, 1990; Aydt et al., 1991; Gennadios and Weller, 1992; Gennadios et al., 1993b, c, d, e, 1994; Gontard et al., 1992, 1993), corn zein (Park and Chinnan, 1990; Aydt et al., 1991; Gennadios et al., 1993d, 1994; Trezza et al., 1992), and soy protein isolate (Gennadios et al., 1993e; Brandenburg et al., 1993). In all the aforementioned studies, film preparation involved casting protein dispersions in water or aqueous ethanol with subsequent drying. A plasticizer, usually glycerol, was added to ensure production of self-supporting films.

In general, protein films are poor water vapor barriers due to the inherent hydrophilicity of protein. However, protein films were found to be very effective oxygen barriers (Brandenburg et al., 1993; Gennadios et al., 1993d). Use as oxygen barrier-providing layers in

multilayer or multicomponent packaging materials, and as coatings for oxygen-sensitive food items in combination with an external efficient moisture barrier constitute examples of potential protein film applications.

Physical and barrier properties of hydrophilic protein films can be significantly influenced by the moisture concentration in the films (Gontard et al., 1993; Gennadios et al., 1994). For example, the tensile strength of corn zein films conditioned for two days at 25° C and 23 or 75% relative humidity was 19.5 and 5.7 MPa, respectively (Gennadios et al., 1994). Similarly, wheat gluten films conditioned for two days at 25° C and 23 or 75% relative humidity had tensile strength values of 5.2 and 2.7 MPa, respectively (Gennadios et al., 1994). According to Watt (1983), water vapor sorption by biopolymers may result in swelling and conformational changes of the macromolecular structure. Consequently, an understanding of the water sorption properties of edible protein films is necessary in order to tailor their application on or in food systems. Experimentally derived moisture sorption isotherms would offer a useful tool for estimating moisture content of a film at equilibrium with a given relative humidity environment.

The present study was undertaken to develop the moisture adsorption isotherms at 25° C of three different types of laboratory-produced grain protein edible films. The three films were made from wheat gluten, corn zein, and a 2.3:1 mixture of wheat gluten and soy protein isolate. Mathematical models of water sorption isotherms were applied on collected experimental data and tested for accuracy of fit.

MATERIALS AND METHODS

PREPARATION OF FILM-FORMING SOLUTIONS

Vital wheat gluten protein (DO-PEP), soy protein isolate (PP 620), and corn zein (F-4000) protein were obtained from ADM Arkady (Olathe, Kans.), Protein

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Technologies International (St. Louis, Mo.), and Freeman Industries (Tuckahoe, N.Y.), respectively. Film-forming solutions for the wheat gluten (WG) films consisted of 15 g WG, 72 mL 95% ethanol, 6 g glycerol, 12 mL 6N ammonium hydroxide, and 48 mL distilled water (Gennadios et al., 1993b, c). Wheat gluten/soy protein isolate (WG/SPI) films had similar composition except that 4.5 g of wheat gluten were substituted with soy protein isolate (Gennadios et al., 1993c). Corn zein (CZ) film-forming solutions were prepared by mixing 10 g CZ, 2 g glycerol, and 65 mL 95% ethanol. All film-forming solutions were heated for 10 min under stirring. Heating rate was adjusted so that at the end of preparation the solutions had a temperature of 75 to 77° C.

FILM CASTING AND DRYING

Film-forming solutions, while still hot, were cast on flat glass plates with a thin-layer chromatography spreader bar (Brinkman Co., New York, N.Y.) set at 1.5 mm. Casting areas on plates were "framed" with a layer of masking tape to restrain solution spreading. Plates with cast solutions were placed in an air-circulating oven (Isotemp, model 338F, Fisher Scientific, Pittsburgh, Pa.) maintained at 35° C. After 15 h, the plates were removed from the oven and the films were peeled off.

FILM THICKNESS

Film thickness was measured with a hand-held micrometer (B.C. Ames Co., Waltham, Mass.) to the nearest 2.54 μ m (0.1 mil). Thickness values for each type of film, representing an average of 30 measurements, were 80 ± 18 , 122 ± 15 , and 132 ± 12 μ m for CZ, WG, and WG/SPI films, respectively.

CHEMICAL ANALYSIS

The three types of film were chemically analyzed by the Department of Agricultural Chemical Services, Clemson University. The dry matter of film samples was determined after freeze-drying at -60° C and 1.33 Pa (10 milliTor). Crude protein was determined with a micro-Kjeldahl method, using a nitrogen conversion factor of 5.70 for WG and WG/SPI films, and of 6.25 for CZ films. Ashing of samples was done in a muffle furnace at 600° C for 4 h. Crude fat contents were determined after extraction with hexane in a Soxhlet apparatus overnight. Carbohydrate contents were estimated by difference. All tests were done in triplicate for each type of film. Results, on a dry basis, are presented in table 1.

DETERMINATION OF MOISTURE ADSORPTION

Constant relative humidity environments were established inside sorbostats, one-pint wide mouth canning jars, using saturated salt solutions. The salts used (table 2) were the 10 different salts recommended by the COST-90 project (Wolf et al., 1984), covering a relative humidity (RH) range from 11.15 to 90.26%. Water activity (a_w) for the films was defined as %RH/100 (Labuza, 1968). Film samples were assumed to have a a_w corresponding to the RH of the sorbostat and to reach an equilibrium moisture content within each sorbostat.

Prior to beginning adsorption tests, film samples were cut into small pieces (approximately 5 $\times$ 5 mm). They were then brought to zero moisture content by freeze-drying at

Table 1. Percent chemical composition (dry basis) of grain protein films*

Film	Crude Protein	Crude Fat	Ash	Carbohydrate
CZ	78.25 $\pm$ 0.32	0.52 $\pm$ 0.26	1.15 $\pm$ 0.11	20.07 $\pm$ 0.35
WG	53.07 $\pm$ 0.52	1.76 $\pm$ 0.33	0.50 $\pm$ 0.04	44.67 $\pm$ 0.56
WG/SPI	54.13 $\pm$ 0.40	1.74 $\pm$ 0.72	1.35 $\pm$ 0.06	42.79 $\pm$ 0.36

* Reported values are means of three replicates plus/minus one standard deviation.

-55° C and 0.67 Pa (5 milliTor) for 24 h (Lyph-Lock 12, Labconco Co., Kansas City, Mo.). Samples of 0.4 g of all films were weighed and placed into aluminum pans, 5 cm in diameter. The aluminum pans which contained the samples were suspended over the sorbate in the sealed sorbostats. The sorbostats were kept inside an environmental chamber maintained at 25° C. The pans had been equilibrated in the sorbostats for two days before their tare weights were recorded.

The weights of samples were recorded to the nearest 0.0001 g after the first two days and every day thereafter until equilibrium. Equilibrium was assumed to have been reached when the change in moisture content of samples was less than 0.001 g water/g dry matter (Labuza, 1984). Equilibrium was observed after one week for the first nine salts (RH range 11.15 to 84.32%). Material deterioration was evidenced, from mold growth and putrid smell, for all samples placed over the salt with the highest RH (90.26%), before equilibrium was reached. Therefore, equilibrium moisture contents at this RH are not reported. Results at equilibrium were reported for each relative humidity as gram water adsorbed/gram dry matter. Adsorption tests were done in triplicate for each type of film at each relative humidity.

MATHEMATICAL MODELS

The four different models for fitting water sorption isotherms of food products which are presented in table 3 were evaluated for accuracy of fit on the experimental data.

MODEL PARAMETERS AND ACCURACY OF FIT

Experimental sorption data for each type of film were fitted to the above mathematical models using a nonlinear regression analysis procedure (NLIN) in a SAS statistical package (SAS Institute, Inc., 1988). The procedure was

Table 2. Preparations of saturated salt solutions at 25° C (Wolf et al., 1984)

Salt	Relative Humidity (%)	Mixing Analogy	
		Salt (g)	Distilled Water (mL)
LiCl	11.15	75	42.5
CH ₃ COOK	22.60	100	32.5
MgCl ₂	32.73	100	12.5
K ₂ CO ₃	43.80	100	45.0
Mg(NO ₃)	52.86	100	15.0
NaBr	57.70	100	40.0
SrCl ₂	70.83	100	25.0
NaCl	75.32	100	30.0
KCl	84.32	100	40.0
BaCl ₂	90.26	125	35.0

Table 3. Moisture sorption isotherm models fitted to experimental adsorption data of edible grain protein films

Name	Model*	Reference
Smith	$M = A - [B \ln(1 - a_w)]$	Smith (1947)
Oswin	$M = A[a_w / (1 - a_w)]^B$	Oswin (1946)
Halsey	$M = [-A / (T \ln a_w)]^{1/B}$	Halsey (1948) [†]
GAB [‡]	$M = (M_o C k a_w) / [(1 - k a_w)(1 - k a_w + C k a_w)]$	Bizot (1984)

* M = equilibrium moisture content (g water/g dry solids); a_w = water activity; T = absolute temperature (K); A = constant; B = constant; M_o = monolayer moisture content (g water/g dry solids); C = Guggenheim constant; and k = constant correcting the properties of the multilayer molecules with respect to the bulk liquid.

[†] As simplified by Chirife and Iglesias (1978).

[‡] Guggenheim-Anderson-de Boer.

used with the iterative modified Gauss-Newton method to regress the residuals onto the partial derivatives of the model with respect to the parameters, until the parameter estimates converge.

The criterion used to evaluate accuracy of fit for each model was the mean relative deviation modulus (P) calculated by the expression:

$$P = (100 / n) \sum (|M_a - M_p| / M_a)$$

where M_a is the actual (experimental) moisture content; M_p is the predicted moisture content; and n is the number of observations. A P value smaller than 5 indicates an extremely good fit; a P value between 5 and 10 represents a reasonably good fit; and a P value greater than 10 shows a poor fit (Lomauro et al., 1985a, b; Gencturk et al., 1986).

RESULTS AND DISCUSSION

MOISTURE ADSORPTION

Experimental data for moisture adsorption at 25° C for CZ, WG, and WG/SPI films are shown in figure 1. Both WG and WG/SPI films had higher equilibrium moisture contents than CZ films at all water activities covered. This observation was mainly attributed to the lower amount of hygroscopic glycerol incorporated in CZ films (20 g/100 g protein) than in WG and WG/SPI films (40 g/100 g

Figure 1—Experimental data points and isotherm curves predicted by the GAB model for moisture adsorption by CZ, WG, and WG/SPI films at 25° C.

Table 4. Mean relative P and constants (M_o , C, and k) of the GAB moisture isotherm equation for moisture adsorption of edible grain protein films at 25° C

Film	a_w Range	n*	P	M_o	C	k
CZ	0.11-0.84	27	7.95	0.1241	0.3456	0.8899
CZ	0.33-0.84	21	3.37	0.1027	0.9333	0.9072
WG	0.11-0.84	27	4.69	0.1052	1.3333	0.9450
WG	0.33-0.84	21	1.21	0.1023	1.4111	0.9490
WG/SPI	0.11-0.84	27	3.44	0.1386	0.8088	0.8959
WG/SPI	0.33-0.84	21	1.75	0.1351	0.8376	0.8993

* Number of experimental data points.

protein). Furthermore, CZ is characterized by a low content of polar (hydrophilic) amino acids and a high content of nonpolar (hydrophobic) amino acids, such as leucine, proline, and alanine (Shewry and Mifflin, 1985). Reportedly, CZ has a higher hydrophobicity, as calculated by amino acid composition, than the prolamin and glutelin protein fractions of WG and other cereals (Belitz et al., 1986).

The WG/SPI films had slightly lower equilibrium moisture contents than WG films at all a_w studied. Water vapor permeability constant values for WG and WG/SPI films, identical to the ones produced in this study, were reported by Gennadios et al. (1993c). Water vapor permeability, which consists of water sorption into and diffusion through a barrier, is a measure of a film's hydrophilicity. Gennadios et al. (1993c) found that the WG/SPI film had a lower, although not statistically significantly different, water vapor permeability constant value than the WG film.

MOISTURE ISOTHERM EQUATIONS

The four moisture isotherm mathematical models (Smith, Oswin, Halsey, and GAB) were fitted to moisture adsorption data for the whole water activity range studied and for specific portions of that range. Calculated model constants and mean relative deviation modulus for each case are shown in tables 4, 5, 6, and 7 for the GAB, Smith, Oswin, and Halsey models, respectively.

For all three types of films, the GAB model showed the best fit over the whole isotherms [see the relative deviation modulus (P) in tables 4 through 7]. It fitted very well for the WG and the WG/SPI films ($P = 4.69$ and 3.44 , respectively) and reasonably well for the CZ film ($P = 7.95$). The fit of the GAB model was even better when applied over the a_w range from 0.33 to 0.84. Chirife et al. (1992) reported that the constant k in the GAB sorption model varies from nearly unity to as low as 0.56 for a large

Table 5. Mean relative P and constants (A and B) of the Smith moisture isotherm equation for moisture adsorption of edible grain protein films at 25° C

Film	a_w Range	n*	P	A	B
CZ	0.11-0.84	27	85.34	-0.0356	0.1411
CZ	0.53-0.84	15	6.74	-0.0843	0.1772
WG	0.11-0.84	27	33.06	-0.0396	0.2361
WG	0.53-0.84	15	5.08	-0.1026	0.2826
WG/SPI	0.11-0.84	27	36.81	-0.0401	0.2239
WG/SPI	0.53-0.84	15	3.56	-0.0966	0.2658

* Number of fitted experimental data points.

Table 6. Mean relative P and constants (A and B) of the Oswin moisture isotherm equation for moisture adsorption of edible grain protein films at 25° C

Film	a _w Range	n*	P	A	B
CZ	0.11-0.84	27	20.05	0.0510	0.9626
CZ	0.11-0.58	18	5.24	0.0485	1.2243
WG	0.11-0.84	27	10.07	0.1095	0.8254
WG	0.11-0.58	18	6.68	0.1086	0.9250
WG/SPI	0.11-0.84	27	13.82	0.1020	0.8307
WG/SPI	0.11-0.58	18	5.25	0.0990	0.9799

* Number of fitted experimental data points.

variety of food constituents, while k values for proteins fall in the range of 0.82 to 0.88. The higher k values calculated for the grain protein films in the present study most likely resulted from the large amount of glycerol (20 to 40% w/w of protein) added to films.

The GAB water sorption isotherm model is the most popular one in the area of food technology, fitting moisture sorption data extremely well for many food materials over a wide range of a_w, up to 0.9 (Bizot, 1984). Lomauro et al. (1985a, b) found that the GAB model gave a very good fit (P < 5) for over 50% of 75 food isotherms representing fruit, vegetable, and meat food products and over 75% of 88 isotherms representing milk, coffee, tea, nuts, oilseeds, spices, and starchy foods. A good fit of the GAB model was also reported for isotherms of wild rice at temperatures ranging from 10 to 43.5° C (Gencturk et al., 1986); raisins and dried apricots at 20° C (Ayranci et al., 1990); and dried fruits (raisins, currants, figs, prunes, and apricots) at 15, 30, 45, and 60° C (Tsami et al., 1990).

The Smith model was proven to fit the high a_w portions of water sorption isotherms of various biopolymers (Smith, 1947). A good fit of the Smith model was also documented for adsorption and desorption isotherms of Virginia-type peanuts above a_w of 0.30 (Young, 1976); desorption isotherms of wheat between a_w of 0.50 and 0.95 (Becker and Sallans, 1956); and adsorption isotherms of cowpeas at the high a_w portion of the curves (Chinnan and Beuchat, 1985). Pixton and Howe (1983) reported that the Smith model gave a satisfactory fit for water sorption curves of several food commodities including field beans, dried peas, dried figs, and rice bran. In the present study, for a_w in the range of 0.53 to 0.84, the Smith model fitted adsorption data of WG (P = 5.08) and WG/SPI (P = 3.36) films well and data of CZ films (P = 6.78) reasonably well (table 5). Fit to the data for the whole range of a_w was very poor.

The Oswin model was found very suitable to describe sorption isotherms of proteins and starchy foods

(Boquet et al., 1978). Lomauro et al. (1985a, b) concluded that this model fitted sorption data for a considerable number of nuts, oilseeds, spices, coffee and tea products, and vegetables quite well. In a study of moisture adsorption by dried bell peppers at 10, 25, and 40° C, the Oswin model gave the best fit among several tested mathematical models (Saputra et al., 1989). For the film sorption data of the present study, the Oswin model gave a good fit (5.24 < P < 6.68) for the a_w range between 0.11 and 0.58 (table 6). The fit on the whole isotherm curves was poor (P = 10 – 20).

Use of the Halsey model was recommended for meats, milk products, and vegetables (Boquet et al., 1978). According to Lomauro et al. (1985b), the same model successfully applied to water sorption data for several nuts, oilseeds, and starchy foods. Iglesias et al. (1975) and Iglesias and Chirife (1976) found that Halsey's model could be used to describe reasonably well the sorption behavior of 69 different food materials in the approximate a_w range of 0.10 to 0.80. Ayranci et al. (1990) reported good fit of the Halsey model to sorption isotherms of dried apricots, dried figs, and raisins. Pixton and Howe (1983) observed a good fit of the Halsey model to moisture sorption curves of field beans, rice bran, rapeseed, linseed, sunflower seed, dried peas, shelled almonds, and lupins. When fitted to the whole a_w range of protein film sorption data in the present study, the Halsey model showed a very poor fit. However, Halsey's model showed an excellent fit (P = 2.55, 4.40, and 4.52 for WG, WG/SPI, and CZ films, respectively) at the high a_w (0.53 to 0.84) portion of the film isotherms (table 7).

CONCLUSIONS

It was demonstrated in the present study that the GAB moisture sorption isotherm model can be successfully applied to moisture adsorption by grain protein films in the water activity range between 0.11 and 0.84. Other models are of value when applied on specific portions of the grain films' adsorption isotherms. The Smith and Halsey models can be used within the high water activity range of 0.53 to 0.84, whereas the Oswin model can be used within the low water activity range of 0.11 to 0.58.

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Table 7. Mean relative P and constants (A and B) of the Halsey moisture isotherm equation for moisture adsorption of edible grain protein films at 25° C

Film	a _w Range	n*	P	A	B
CZ	0.11-0.84	27	48.08	0.0535	0.8618
CZ	0.53-0.84	15	4.52	0.0479	0.9333
WG	0.11-0.84	27	29.25	0.0767	0.9900
WG	0.53-0.84	15	2.55	0.0707	1.0678
WG/SPI	0.11-0.84	27	33.78	0.0723	0.9850
WG/SPI	0.53-0.84	15	4.40	0.0652	1.0799

* Number of fitted experimental data points.

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