

## Water Sorption Properties of Extruded Zein Films

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Water interactions in extruded zein films were investigated through moisture sorption isotherms. Sorption isotherms of zein products were affected by composition and structure morphology. Zein powder showed moisture sorption hysteresis, which was not observed in extruded samples. Extruded samples held less moisture than zein powder, while films containing oleic acid showed further reduction in moisture uptake. Brunauer, Emmett, and Teller (BET) and Guggenheim, Anderson, and De Boer (GAB) models fitted well the moisture sorption isotherms of zein products. Monolayer values estimated by BET and GAB models were consistent with predictions based on zein structural models. Water vapor permeability (WVP) of zein films was affected by the relative humidity of testing environment. Higher relative humidity resulted in higher WVP.

**KEYWORDS:** Zein; extrusion; films; moisture sorption isotherm; water vapor permeability

### INTRODUCTION

Agricultural or biobased alternatives to petroleum-derived packaging materials are of increasing interest. These “agro-packaging” materials include polysaccharides, proteins, and lipids. Various proteins from different sources have been studied for their potential as edible food packaging (1), including zein, wheat gluten, soy protein, cottonseed protein, whey protein, casein, fish myofibrillar protein, and egg white protein.

The film forming properties of zein have been long recognized. They are the basis for zein commercial applications (2–4). Formation of free-standing zein films for food packaging is currently the focus of intensive research and development (5–9). Zein films are generally formed by solution casting. Zein, plasticizers, and other agents are dissolved in an appropriate solvent and the solution is cast on a flat nonstick surface from where a free-standing film is peeled off after the solvent evaporates. However, solution casting is a process difficult to scale up. Extrusion is a standard method used in the plastics industry to compound and form petroleum-based polymers, such as low-density polyethylene (LDPE); however, extrusion forming of protein films has been a challenge to researchers and few reports have been published. Ha and Padua (10) reported the slit-die extrusion of zein plasticized with oleic acid. They determined an optimum plasticization level of 0.7 g of oleic acid/g of zein for their process. The same formulation was later used by Wang and Padua (11) to prepare films by blown film extrusion.

Physical and barrier properties of protein films are significantly influenced by moisture content. In general, protein films are poor moisture barriers due to the hydrophilic nature of

proteins, but they are good barriers to gases such as oxygen and carbon dioxide. Water vapor permeability (WVP) values of protein films are normally 2–4 orders of magnitude higher than that of LDPE (12). Alcohol-soluble proteins, such as zein, wheat gluten, and fish myofibrillar proteins, form films of relatively low WVP when compared to other proteins (12). Plasticization affects WVP. Zein films containing no plasticizers had lower WVP values than those plasticized with glycerol or mixtures of glycerol with poly(ethylene glycol) or poly(propylene glycol) (13–15). Relative humidity (RH) markedly affects WVP and oxygen permeability of protein films. Moisture has a plasticizing or swelling effect on polymers, resulting in increased gas permeability (16). Lai and Padua (9) observed increasing WVP values as the RH of the test environment increased from 53% to 98%. Protein films have low oxygen permeability at low to intermediate RH. Oxygen permeability values are comparable to those of modest oxygen barriers, such as polyesters, and approaching those of the best oxygen barriers, ethylene–vinyl alcohol copolymer and poly(vinylidene chloride) (12). However, oxygen permeability of protein films significantly increases with increased RH. Oxygen permeability of collagen films (17) increased from 29 to 890  $\text{cm}^3 \cdot \mu\text{m} / \text{m}^2 \cdot \text{day} \cdot \text{kPa}$  when the testing RH changed from 63% to 93%. Water sorption properties of zein films were investigated in the present study. Zein films were plasticized with oleic acid and formed by extrusion blowing.

### MATERIALS AND METHODS

**Materials.** Zein, regular-grade, protein content 90%, water content 4.7%, was used as received (F4000, Freeman Industries Inc., Tuckahoe, NY). Oleic acid ( $\text{C}_{18:1}$ ) 90% (Aldrich Chemical Co., Milwaukee, WI), was used as plasticizer. Distilled monoglycerides (DMG-130, Archer Daniels Midland Co., Decatur, IL) were used as emulsifiers. The solvent used was ethanol, technical-grade (Midwest Grain Products, Pekin, IL).

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Salts used to control relative humidity inside sorbostats were anhydrous calcium sulfate, lithium chloride, potassium acetate, magnesium chloride, and calcium nitrate, reagent-grade (Fisher Scientific, Fair Lawn, NJ); potassium carbonate, reagent-grade (J. T. Baker Chemical Co., Phillipsburg, NJ); and ammonium nitrate, sodium chloride, potassium chloride, and potassium sulfate, reagent-grade (Sigma-Aldrich, Inc., St. Louis, MO).

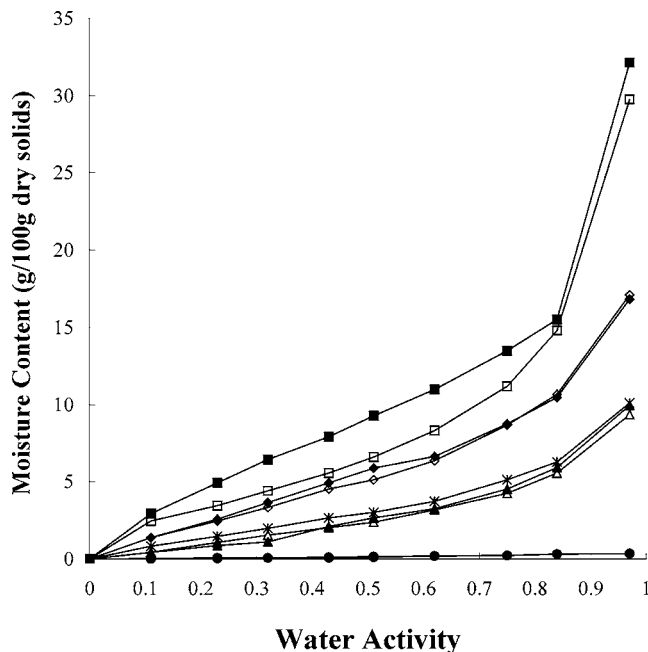
**Blown Extrusion of Zein Films.** Zein, oleic acid (70 g/100 g of zein), and DMG-130 (5 g/100 g of zein) were dissolved in 75% ethanol at 60–70 °C and stirred for 10 min. The solution was then poured into an ice–water mixture to form a precipitate, which was collected and kneaded in a Farinograph (Type FT3, Brabender Corp., Rochelle Park, NJ) for 30 min to form a cohesive mass (resin). Zein resin was extruded at room temperature in a single-screw extruder (Model EPL-V501, C.W. Brabender, Hackensack, NJ) for 3–4 cycles to prepare an extrudate suitable for blowing film. A blowing head was then attached to the extruder. Temperature at the three zones of the extruder barrel was kept at 25, 25, 35 °C, and the blowing head was at 45 °C. After exiting the extruder barrel, the resin flowed into the blowing head channel and was forced upward, forming a tube. Air at higher than atmospheric pressure was introduced into the interior of the zein tube. The resin expanded from the base after coming out of the blowing head, forming an expanded tube, which was clasped by hand at the top. After being allowed to dry at room conditions, it formed a glossy and flexible film, 0.15–0.25 mm in thickness (see ref 11).

**Slit-Die Extrusion of Zein Sheets.** Zein sheets were prepared by single-screw extrusion of the resin prepared as described above but without added plasticizers. The resin was passed through a slit die (50 × 1.3 mm) to form a ribbon. The three zones of the extruder barrel were kept at room temperature. The ribbon was pulled off and collected by hand. It was dried at ambient temperature and kept in sealed opaque containers prior to testing to protect it from oxygen and light. The thickness of dried ribbons ranged from 0.3 to 0.4 mm.

**Moisture Sorption Isotherm (MSI) Determination.** A series of environments with relative humidity (RH) ranging from 0% to 97% were established in sorbostats by salt or saturated salt solutions. Salts used to control relative humidity inside sorbostats were anhydrous calcium sulfate (RH = 0%), lithium chloride (RH = 11%), potassium acetate (RH = 23%), magnesium chloride (RH = 32%), potassium carbonate (RH = 43%), calcium nitrate (RH = 51%), ammonium nitrate (RH = 62%), sodium chloride (RH = 75%), potassium chloride (RH = 84%), and potassium sulfate (RH = 97%). All sorbostats were kept at 25 ± 1 °C. Samples, placed in aluminum pans, were kept inside the sorbostats for 3 weeks. After that period, samples were assumed to have reached equilibrium at water activity ( $a_w$ ) values equal to the corresponding RH of the sorbostats. Adsorption and desorption were tested in triplicate for each type of sample. For adsorption determinations, samples were preconditioned for 2 weeks at 0% RH in a sorbostat containing anhydrous calcium sulfate. For desorption determinations, samples were preconditioned at 100% RH in a sorbostat containing distilled water. Moisture content was measured according to AACC Method 44-15A (18). Samples were initially weighed and dried at 103 °C for 2 h, cooled in a desiccator containing anhydrous calcium sulfate, and weighed again. The samples were put back into the oven for another 2 h and the above steps were repeated until the change in calculated moisture was less than 0.2%. Moisture content was expressed in grams of water per 100 grams of dry solid. For the MSI determination of oleic acid, moisture sorption was determined by weight difference between the initial and equilibrated samples.

**BET and GAB Models.** The Brunauer, Emmett, and Teller (BET) (19) and Guggenheim, Anderson, and De Boer (GAB) (20–22) models were fitted to experimental moisture data. Nonlinear regression analysis was applied with an 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 converged. The criterion used to evaluate the goodness of fit was the mean relative deviation modulus ( $p$ ) calculated by (7)

$$p = \frac{100}{n} \sum_{i=1}^n \frac{|M_a - M_p|}{M_a} \quad (1)$$



**Figure 1.** Moisture sorption isotherms of zein products. Zein powder adsorption (□); zein powder desorption (■); zein sheet adsorption (◇); zein sheet desorption (◆); zein–oleic acid film adsorption (△); zein–oleic acid film desorption (▲); oleic acid adsorption (○); oleic acid desorption (●); calculated isotherm (\*).

where  $M_a$  is the experimental moisture content,  $M_p$  is the predicted moisture content according to the models, and  $n$  is the number of observations. A  $p$  value below 5 indicates an extremely good fit. A  $p$  value between 5 and 10 means a fairly good fit, while a  $p$  value greater than 10 gives a poor fit.

**WVP Measurement.** WVP of blown zein films was monitored according to ASTM E 96-95 desiccant method (23). Test cells covered and sealed by test films were placed in a sorbostat, which was maintained at 51% RH by a saturated solution of calcium nitrate collected at the bottom of the sorbostat or at 100% RH by distilled water. Anhydrous calcium sulfate was used to maintain a 0% RH atmosphere inside the test cell. The sorbostat was stored at a controlled temperature of 25 ± 1 °C. Water vapor transmission (WVT, grams per square meter per day), was determined by

$$WVT = \frac{G}{tA} \quad (2)$$

where  $G$  is the weight change of test cells (grams),  $t$  is the time (days) during which  $G$  occurred, and  $A$  is the test area (square meters). Permeability was calculated according to the formula:

$$P = \frac{WVT}{S(R_1 - R_2)}L \quad (3)$$

where  $P$  is permeability (g·mm/ m<sup>2</sup>·d·kPa),  $S$  is saturation vapor pressure at test temperature (kilopascals),  $R_1$  is relative humidity in the test sorbostat expressed as a fraction,  $R_2$  is relative humidity inside the test cell expressed as a fraction, and  $L$  is the film thickness (millimeters).

## RESULTS AND DISCUSSION

**Moisture Sorption Isotherms.** Eight sorption curves were determined: adsorption and desorption of zein powder, adsorption and desorption of extruded zein sheets without plasticization (termed zein sheet), adsorption and desorption of extrusion blown zein films plasticized with oleic acid (termed zein–oleic acid film), and adsorption and desorption of oleic acid. Sorption isotherms are shown in **Figure 1**. Zein powder shows a

sigmoidal isotherm. A sharp increase in moisture content is observed from 0 to 0.11  $a_w$ , followed by a steady increase in moisture until 0.75–0.84  $a_w$  before moisture content abruptly increases again. This behavior was believed to be due to sample morphology. Zein powder is formed by protein aggregates with multiple pores and capillaries that are able to hold droplets of water. Moreover, zein powder showed moisture sorption hysteresis; that is, more water is held at the same  $a_w$  for desorption than for adsorption curves (24). Upon desorption, water could be trapped and held in crevices and capillaries, resulting in higher moisture content than in adsorption. Furthermore, high moisture uptake by zein powder could induce protein swelling, exposing previously occluded polar amino acid residues to water. Both capillary effects and protein swelling may have contributed to moisture sorption hysteresis in zein powder. Neither sigmoidal curves nor hysteresis was observed in other samples.

Extruded products adsorbed less water than zein powder. Moisture content of extruded sheets without plasticization rose steadily from 0 to 0.84  $a_w$  and sharply increased afterward. Moisture content of sheets was substantially lower than that of zein powder through the  $a_w$  range studied. The difference may be attributed to structural differences between the two samples. Since only aqueous ethanol was added to zein during the preparation of resin for extrusion and ethanol was removed by subsequent steps, the composition of zein powder and extruded sheets was identical. The resin formation process and subsequent extrusion may have modified the morphological structure of zein aggregates and compressed the zein mass into a compact solid, removing pores and capillaries and thus lowering its moisture sorption capacity.

Moisture adsorption of zein films plasticized with oleic acid was lower than of zein sheets without plasticization, indicating that the incorporation of hydrophobic oleic acid prevented moisture sorption by zein films. **Figure 1** shows that the moisture sorption capacity of oleic acid at all water activity values can be neglected when compared to that of other samples. Wei and Baianu (25) studied the moisture sorption of cast zein films prepared with different plasticizers. They observed that glycerol-plasticized films had higher water sorption capacity than zein powder or unplasticized zein films. This was attributed to the hydrophilic character of glycerol. Further investigation showed that increasing the hydrophilic character of plasticizers increased the amount of water adsorbed by films. Glycerol showed the highest affinity for water, followed by poly(ethylene glycol) and lactic acid. Lawton (26) ranked the ability of different plasticizers to enhance the water adsorption capacity of zein films as glycerol > triethylene glycol > levulinic acid > poly(ethylene glycol) > no plasticizer > dibutyl tartrate > oleic acid, which is consistent with the results in this work and those of Wei and Baianu (25). **Figure 1** also shows a calculated sorption isotherm representing the zein contained in zein sheets. Each of the experimental values of the isotherm for sheets prepared without plasticization was multiplied by 0.59 to factor out the mass fraction corresponding to oleic acid (41%). The calculated isotherm nearly overlaps that of blown films, indicating that the moisture sorption capacity of zein films is proportional to their zein content. The additive property of the sorption isotherm suggested that oleic acid was not strongly attached to the zein surface in extruded films.

Moisture content of zein products increased sharply at  $a_w$  values close to 1, as seen in **Figure 1**. High moisture content had a readily noticeable effect on their mechanical properties. Films seemed more flexible when kept above 75% RH and

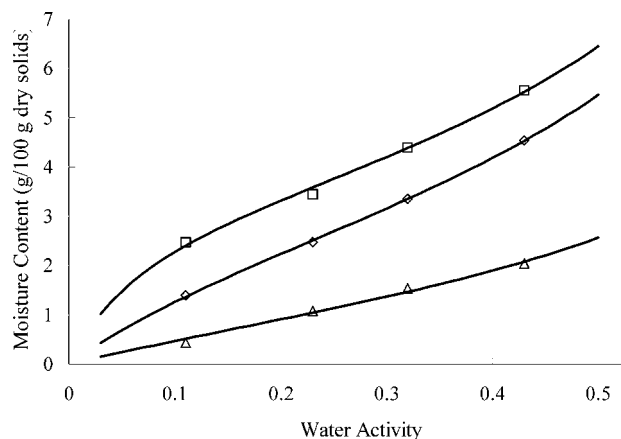
**Table 1.** BET and GAB Parameters for Sorption Isotherms of Zein Products

| sample                         | model | $a_w$ range | $P$  | estimated values of parameters <sup>a</sup> |                 |                 |
|--------------------------------|-------|-------------|------|---|-----------------|-----------------|
|                                |       |             |      | $M_0$                                       | $c$             | $k$             |
| zein powder                    | BET   | 0.11–0.43   | 1.83 | $3.47 \pm 0.10$                             | $13.0 \pm 2.1$  |                 |
|                                | GAB   | 0.11–0.84   | 2.21 | $4.08 \pm 0.13$                             | $9.78 \pm 1.3$  | $0.87 \pm 0.01$ |
| zein sheet (no plasticization) | BET   | 0.11–0.43   | 1.15 | $3.31 \pm 0.06$                             | $4.69 \pm 0.27$ |                 |
|                                | GAB   | 0.11–0.84   | 1.65 | $4.16 \pm 0.27$                             | $4.67 \pm 0.59$ | $0.77 \pm 0.02$ |
| zein film (41% oleic acid)     | BET   | 0.11–0.43   | 7.36 | $1.72 \pm 0.21$                             | $2.94 \pm 0.89$ |                 |
|                                | GAB   | 0.11–0.84   | 4.71 | $2.12 \pm 0.22$                             | $3.14 \pm 0.56$ | $0.80 \pm 0.03$ |

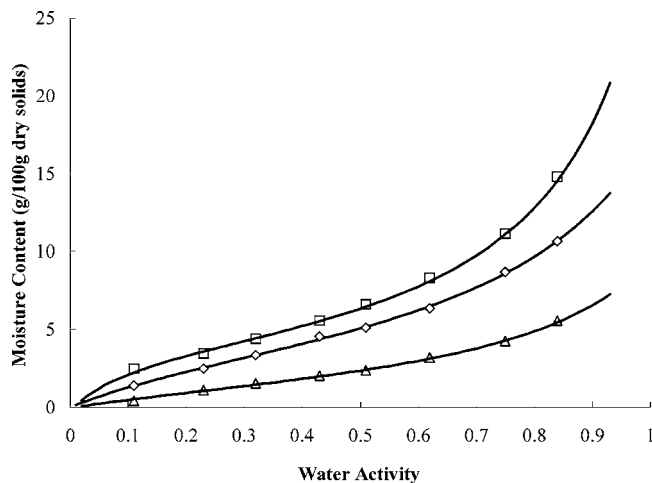
<sup>a</sup> Mean  $\pm$  standard deviation.

became highly stretchable at 97% RH. Lawton (26) prepared a series of cast zein films using different plasticizers. He reported that films containing oleic acid did not show any increase in elongation-at-break below 93% RH. di Gioia et al. (27) reported that when water content increased from 0 to 22.6%, molded corn gluten meal (CGM) materials at plasticization level of 20 g of oleic acid/100 g of dry CGM lowered their tensile strength from 24.1 to 5.6 MPa and their elastic modulus from 1.67 down to 0.13 GPa, while elongation at break increased from 2% to 52.3%. This effect was attributed to the lowering of the glass transition temperature ( $T_g$ ) caused by water plasticization.  $T_g$  measurements confirmed that, at 20 °C and above 12% water content, oleic acid-plasticized CGM was in a rubbery state and could be highly extended with a small force.

**BET and GAB Models.** BET and GAB model parameters, evaluated from nonlinear regression analysis, for moisture adsorption of zein powder, extruded zein sheets without plasticization, and zein films plasticized with oleic acid are shown in **Table 1**. It is noted that only moisture adsorption curves were fitted with BET and GAB equations. Both BET and GAB models fitted the isotherm data for zein products with  $p$  values below 5, except for the BET fit to zein films. However, a  $p$  value of 7.36 indicates that BET still fits zein films fairly well. **Figures 2** and **3** show the curve-fitting results; points represent experimental data and lines represent the fitted curves. Over 70 equations have been proposed for modeling moisture sorption isotherms (24), among which BET and GAB model are the most frequently used. The BET model (19) is based on the assumption that a fixed number of active sorption sites are independently and randomly distributed throughout the sorbent solid. The first layer of sorbate molecules or monolayer is thought to have a much stronger interaction with the sorbent sites than the second and following water layers. The main limitation for this model is that it fits only between 0.05 and



**Figure 2.** Fitting of moisture adsorption curves of zein products with BET model. Zein powder ( $\square$ ); zein sheet ( $\diamond$ ); zein-oleic acid film ( $\triangle$ ).



**Figure 3.** Fitting of moisture adsorption curves of zein products with GAB model. Zein powder ( $\square$ ); zein sheet ( $\diamond$ ); zein-oleic acid film ( $\triangle$ ).

0.45  $a_w$ . The expression for the BET model is

$$M = \frac{M_0 c a_w}{(1 - a_w)(1 - a_w + c a_w)} \quad (4)$$

where  $M$  is the equilibrium moisture content on a dry basis,  $M_0$  is the monolayer moisture content,  $a_w$  is water activity, and  $c$  is a constant related to the net heat of sorption. The GAB equation (20–22) extended the BET application to  $a_w$  ranging from 0.1 to 0.90. The GAB equation has the form

$$M = \frac{M_0 c k a_w}{(1 - k a_w)(1 - k a_w + c k a_w)} \quad (5)$$

where  $M$  is the equilibrium moisture content on dry basis,  $M_0$  is the monolayer moisture content,  $a_w$  is water activity,  $c$  is the Guggenheim constant, which is related to the heat of sorption of the first layer, and  $k$  is a parameter related to the total heat of sorption.

Isotherm parameters show differences between BET and GAB models, as seen in **Table 1**. The BET monolayer value for zein powder is 3.47 g/100 g of dry solid, slightly lower than that calculated by GAB, 4.08 g/100 g of dry solid. The same trend can be seen in films and sheets: the GAB monolayer is slightly higher than that calculated by BET. Timmermann et al. (28) noted that the BET monolayer is always less than the GAB value and gave a mathematical explanation for it. The monolayer value of zein powder was similar to that of zein sheets, despite the differences in structure morphology between the two samples, indicating that excess water adsorbed by zein powders compared to extruded sheets was held in the interstices of zein aggregates. Pauling (29) believed that in the monolayer of water sorbed onto proteins every molecule of water corresponded to a polar group on the amino acid side chains of the protein. In that case, monolayer moisture is determined only by the active sites of the solid capable of binding water. Zein powder and extruded sheets have the same chemical composition, and therefore the same number of water binding sites, resulting in similar monolayer values for the two samples. However, when oleic acid was incorporated into the system, the number of active sites relative to the mass of the film was reduced. **Table 1** shows that the monolayer value of films containing oleic acid, fitted by both BET and GAB equations, decreased substantially with respect to samples without plasticization.

**Table 2.** Amino Acid Composition of Zein (from Reference 27)

| class     | amino acid    | amino acid content            |                 |                   |
|-----------|---------------|-------------------------------|-----------------|-------------------|
|           |               | molecular weight <sup>a</sup> | g/100 g of zein | mol/100 g of zein |
| nonpolar  | glycine       | 57.03                         | 0.7             | 0.012             |
|           | alanine       | 71.04                         | 8.3             | 0.12              |
|           | valine        | 99.07                         | 3.1             | 0.031             |
|           | leucine       | 113.09                        | 19.3            | 0.170             |
|           | isoleucine    | 113.09                        | 6.2             | 0.055             |
|           | phenylalanine | 147.07                        | 6.8             | 0.046             |
|           | tryptophan    | 186.09                        | 0               | 0                 |
|           | proline       | 97.06                         | 9               | 0.09              |
|           | -OH           | serine                        | 87.04           | 5.7               |
| threonine |               | 101.16                        | 2.7             | 0.027             |
| tyrosine  |               | 163.07                        | 5.1             | 0.031             |
| -S        | methionine    | 131.13                        | 2               | 0.015             |
|           | cysteine      | 103.10                        | 0.8             | 0.008             |
| basic     | lysine        | 128.11                        | 0               | 0                 |
|           | arginine      | 156.38                        | 1.8             | 0.012             |
|           | histidine     | 137.07                        | 1.1             | 0.008             |
| acidic    | aspartic acid | 115.58                        | 0               | 0                 |
|           | asparagine    | 114.58                        | 4.5             | 0.039             |
|           | glutamic acid | 129.06                        | 1.5             | 0.012             |
|           | glutamine     | 128.06                        | 21.4            | 0.170             |

<sup>a</sup> This column is calculated by subtracting 18.02 (molecular weight of water) from the molecular weight of each amino acid.

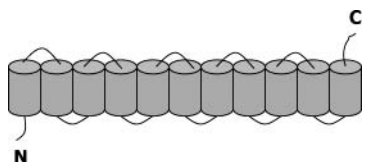
On the basis of the assumption by Pauling (29) that monolayer water on proteins corresponds to one water molecule attached to every polar group of the amino acid side chains, monolayer values could be predicted from the amount of polar groups in zein. The amino acid composition of zein (30) is shown in **Table 2**. Zein has 0.38 mol of polar groups per 100 g of zein, thus yielding a monolayer value of 6.97 g of water/100 g of zein. This result is of the same order of magnitude as the values calculated from BET (3.47 g of water/100 g of zein) and GAB (4.08 g of water/100 g of zein) equations. However, BET and GAB monolayer values are lower than predicted from the total amount of polar groups. This result may be due to the fact that not all polar groups in zein are exposed to water; some are coiled inside the molecule and therefore unable to bind water.

The hydrophobic character of zein is due to its large proportion of hydrophobic amino acids (see **Table 2**). Among polar residues, glutamine is the most abundant. Based on X-ray scattering measurements of zein in solution, Matsushima et al. (31) proposed a structural model related to that of Argos et al. (32). Zein showed sequence homology: N-terminal contains 35–37 amino acids, C-terminal has 8 amino acids, and the central domain consists of 9 or 10 repetitive segments. These repetitive domains contain blocks of 14–25 amino acid residues with an average length of 19 and 20 amino acids (33, 34). The model by Argos et al. (32) consisted of 9 or 10 helical segments, corresponding to the above-mentioned repetitive domains, folded in antiparallel fashion. Helical segments were linked by glutamine-rich turns and held in place by helix-to-helix hydrogen bonds. In the model by Matsushima et al. (31), the helical segments were aligned to form a compact prism with a portion of the N-terminus forming an additional helical segment at the end of the prism. **Figure 4** shows the zein structural model by Matsushima et al. (31), where the outside surfaces of the helical segments contain mostly hydrophobic residues while the top and bottom sides of the molecule contain the hydrophilic glutamine loops. Accordingly, when zein is exposed to a moist environment, water interacts with glutamine residues while most

**Table 3.** Water Vapor Permeability of Zein Films

| film description     | plasticization <sup>a</sup> | test conditions   | WVP (g·mm/m <sup>2</sup> ·d·kPa) | ref |
|----------------------|-----------------------------|-------------------|----------------------------------|-----|
| extruded film        | zein:OA = 1.4:1             | 25 °C, 100/0% RH  | 3.70 ± 0.31 <sup>b</sup>         |     |
| extruded film        | zein:OA = 1.4:1             | 25 °C, 51/0% RH   | 1.92 ± 0.08 <sup>c</sup>         |     |
| stretched resin film | zein:OA = 2:1               | 25 °C, 53/0% RH   | 1.05 ± 0.07                      | 9   |
| cast film            | zein:OA = 2:1               | 25 °C, 53/0% RH   | 1.58 ± 0.27                      | 9   |
| cast film            | zein:(PEG + GLY) = 5.9:1    | 25 °C, 50/100% RH | 47                               | 39  |
| cast film            | zein:(PEG + GLY) = 2.6:1    | 25 °C, 50/100% RH | 107                              | 39  |

<sup>a</sup> OA = oleic acid; PEG = poly(ethylene glycol); GLY = glycerol. <sup>b</sup> Mean ± standard deviation; film thickness = 0.139 ± 0.006 mm. <sup>c</sup> Mean ± standard deviation; film thickness = 0.136 ± 0.002 mm.

**Figure 4.** Zein structural model proposed by Matsushima et al. (31).

other polar amino acids are forming intramolecular hydrogen bonds. When only the glutamine residues are used to calculate the monolayer value, the result is 3.06 g/100 g of dry solid, which is very close to the values determined from BET and GAB sorption models. Moisture sorption determinations were in agreement with the structural model.

The constant  $c$  of the BET model is related to the heat of sorption of water. However, by postulating that the state of water molecules in the second and higher layers is the same with respect to each other but different from that in the liquid state, the GAB model introduced a second well-differentiated stage in the sorption sequence. On the basis of this assumption, another constant  $k$  was introduced in the model. Timmermann et al. (28) also noted that the energy constant of BET is always larger than the product of energy constants  $c$  and  $k$  in GAB. This is true for all zein products shown in **Table 1**. Unlike the parameter  $M_0$ , the  $c$  value of sheets appeared to be closer to that of films than to that of zein powder. This might be an effect of processing; zein films and sheets were both extruded products. Extrusion processing may have resulted in a distinct structure morphology leading to a different heat of sorption from that of zein powder. It was also noted that the standard error for the constant  $c$  was higher than those of  $M_0$  and  $k$ , consistent with the results of Timmermann et al. (28). The value of  $k$  was similar for the three zein products. This is reasonable since  $k$  is a factor correcting the properties of multilayer molecules with respect to the bulk liquid. Chirife et al. (35) reported that  $k$  values for proteins fall in a narrow range between 0.82 and 0.88, with an average of 0.84, which is close to the  $k$  values obtained for zein products.

**WVP of Zein Films.** WVP values of extrusion blown zein films are listed in **Table 3**. WVP values for other films are listed for comparison. WVP of extrusion blown films was affected by RH. As shown in **Table 3**, WVP of films measured at 100/0% RH was nearly twice as high as that measured at 50/0% RH. Lai and Padua (9) reported similar results when they examined WVP of cast zein films plasticized by oleic acid, increasing RH led to increasing WVP. This was attributed to the plasticization effect of water on protein films. Water is a strong plasticizer for biopolymers, although not necessarily a good solvent. The mechanism of water plasticization was believed to be governed by the free volume theory (36). Water increases the polymer free volume, allowing the segments of polymeric chains to increase their mobility (37, 38). Higher segment mobility resulted in higher WVP. **Table 3** shows that

addition of hydrophilic plasticizers increased WVP of zein films with respect to films plasticized with oleic acid. For films formulated with oleic acid, the processing methods applied, i.e., extrusion or casting, had no major effect on WVP.

In conclusion, water interactions in zein products are affected by composition and structure morphology. Zein powder had high moisture adsorption and showed hysteresis, believed to be due to the effect of pores and capillaries in the aggregated structure of the powder. Extrusion led to a more compact structure, resulting in the elimination of hysteresis and lower levels of moisture sorption. Plasticization with oleic acid further reduced moisture sorption of films proportionally to their zein content. Sorption isotherms of zein products were fitted with the BET and GAB models. The zein monolayer estimated from the isotherms was in close agreement with the monolayer value calculated from the number of polar amino acids at the zein surface, according to the structural model presented by Argos et al. (32) and Matsushima et al. (31). The consistency of the isotherm monolayer with the value calculated from the structural model may be interpreted as evidence toward the validity of the structural model. WVP of extrusion blown films was affected by relative humidity. High relative humidity resulted in high WVP due to the plasticization effect of water on zein. WVP of zein films plasticized with oleic acid was lower than that of films containing hydrophilic plasticizers, as reported in the literature. WVP was affected to a larger extent by product composition than by the film forming process. The choice of plasticizer is a major factor in determining water adsorption of zein films.

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