Physicochemical Properties of Plasticized Corn Zein Films: NMR and Adsorptivity Studies

W. Wei and I.C. Baianu *

*Department of Food Science and Human Nutrition, ACES College,
*also Department of Nuclear Engineering, University of Illinois at Urbana,
905 S. Goodwin Ave., 260 Bevier Hall, Urbana, IL 61801, USA

SUMMARY: Water sorption isotherms of corn zein-based films are typical sigmodial curves. The presence of plasticizers greatly increase film water sorption at intermediate and high water activities. The presence of a plasticizer increases water vapor permeabilities of zein films. The extent of such effect depends on the plasticizer type and concentration used for film formation.

Introduction

Plasticized polymeric films of the hydrophobic proteins extracted from corn, the zeins, are potentially important for packaging and other industrial applications. A suitable choice of plasticizer type and concentration is essential for adequate performance of such films in industrial applications.

Materials and Methods

A special grade corn zein (Lot #F5000515C) was obtained from Freeman Industries of Tuckahoe, NY. The moisture content was 6.9% as determined by vacuum oven drying at 60 ^{o}C and 20 in Hg for 24 hour. 85% lactic acid, glycerol, calcium chloride, magnesium chloride, sodium chloride, ammonium sulfate, potassium nitrite, sodium monophosphate were purchased from Fisher Scientific (Fair Lawn, NJ). Potassium chloride, sucrose, zinc sulfate heptahydrate were purchased from EM Science (Gibbstown, NJ). Sodium bromide, lithium chloride were purchased from Mallinckrodt Inc. (St. Louis, MO). Polyethylene glycol was purchased from Aldrich Chemical Company Inc. Ethanol (90%, reagent) was purchased from Midwest Solvent Co. of Pekin, IL.

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Sample Preparation

Corn zein film forming solutions were prepared by dissolution of 2g corn zein into 6.5 ml 90% ethanol. 0.5g glycerol was then added to the solution under magnetic stirring as plasticizer. Other two plasticizers studied were lactic acid and polyethylene glycol 200(PEG) and were employed for comparison at the same concentration (25g/100g zein). Prior to casting, the solutions were allowed to stand for hand-an-hour in order to eliminate bubbles. The solutions were then case onto a 1.1cmX1.9cm Teflon sheet supported by a glass plate, following a method developed in our laboratory. 2g total solids per sheet was applied to minimize thickness variation between treatments. The solutions were spread evenly and allowed to dry overnight at room temperature. Dried film could be easily peeled intact from Teflon sheet. Films were then stored in desiccator for the further measurements.

Water Sorption Measurements

Water sorption isotherm determinations were carried out through measurement of equilibrium vapor pressures in paralleled with the weight loss or gain of test specimens that were recorded as a function of equilibrium time. Equilibrium was considered to be reached when no more weight change occurred for longer than three days. Initially, all samples were dried in a vacuum desiccator at room temperature over P_2O_5 for at least one week to ensure "complete" dryness. Triplicate "dry" samples were then placed in small tightly closed containers. Each container was in equilibrium either with dilute sucrose of with saturated salt solutions of known water activity (Aw) as shown in Table 1 (AOAC standards). Temperature was maintained at $20\pm1^{o}C$ in a controlled temperature room. Samples were accurately weighted to the nearest 0.0001g. When the weight gain was less than 0.1% for three consecutive weightings at 1 day interval, the samples were considered to reach equilibrium. Equilibrium moisture content were then calculated from weight gain. The moisture content were then plotted against water activities to obtain the water sorption isotherm.

^{1}H NMR Measurements

All films were cut into 5 X 40 mm strips. The strips were inserted into capillary tubes which were put into 100 mm NMR tubes (Wilmad Class Co., Buena, NJ) before the

NMR measurements. The samples were then equilibrated against different relative humidity (RH) standards as described for the moisture sorption isotherm determinations. After reaching equilibrium, ^1H NMR measurements were carried out at the School of Chemistry NMR Facility on a GN300 narrow bore multinuclear spectrometer (General Electric Co., NMR Instruments, Freemont, CA) equipped with a 7.05 Telsa superconducting magnet operating at 300.511-MHz proton resonance frequency. Fourier transforms were performed with GE software on-line with a Nicolet 1280 dedicated computer using an FT size of 8K points for the 8K data points acquired.

Film Thickness Measurements

The thickness of films was measured with an AMES Dial Gauge (No.2A) to the nearest 0.001mm. Five random positions around the film were taken for each sample. Average film thicknesses were used in the WVP calculation.

Water Vapor Permeability Determination

Water vapor permeability (WVP) of films was determined gravimetrically at 20±1°C using a modified ASTM (1983) procedure. The glass permeation cells were 2.4 cm (i.d.) by 2.8 cm (o.d.) by 2.4 cm height with an exposed film area of 4.5cm^2. The films were sealed in the permeation cells using a silicone sealant (High Vacuums Grease, Dow Corning, Midland, MI). Permeation cells were stored in small desiccators each maintained at 0% RH (low water vapor pressure) using anhydrous calcium sulfate. Distilled water was added to the permeation cells in order to generate 100%RH atmosphere underneath the films (high water vapor pressure). In order to examine the effects of different water activities on films' water vapor permeability, different salt solutions were used instead of water to reach desired RH gradient across the films Permeability was calculated with the following equation:

$$WVP = \frac{k\,\delta x}{A\,\delta P} \quad \frac{gm}{m^2 s\,Pa} \qquad (1)$$

Where δx is the film thickness, A is the area of exposed film δP is the water vapor pressure differential across the film and k is the slope the plot of weight loss of the permeation cell versus time. The steady-state was reached within 24 hours. The weight loss of the permeation cells were recorded for 7 days at 24 hour intervals to nearest 0.0001g. Four replicates of each film were tested. The slopes were calculated by linear regression and correlation coefficients for all data were 0.99 or greater.

Conditioning

Physical and gas transmission properties of protein based films are influenced by temperature and relative humidity. These conditions must be standardized before a reliable comparison can be made of different materials[1]. Prior to water vapor permeability, all film specimens were conditioned for two days in a desiccator containing a NaBr saturated solution at 20±1°C (59% RH).

Microcomputer Analysis of Experimental Data

The analysis of water sorption isotherm data employed SYSTAT nonlinear regression programs. The program were run on a Macintosh II microcomputer using a Quasi-Newton algorithm (SYSTAT, V.3.1 & V.5.1) in order to obtain confidence intervals for the iterated parameters. The loss function represents the Root Mean Square (RMS) Deviation, indicates whether the fitting is acceptable or not. The two-step protein aggregation model was used to fit the experimental sorption isotherm data from protein powders and films. Data were analyzed by minimizing the sum of squares of the residuals (SSR) and the RMS values for a given set of n and m values in equation 2. After obtaining the best fit values for the A and K parameters, the n and m were fixed to new integer values, one at a time, and the A and K parameters were re-iterated. The reported values are those that yielded the overall lowest RMS values. Two-step protein aggregation model[2] was employed in this nonlinear regression analysis.

The residual sum of squares (SS) was calculated as follows:

$$SS = \sum [(Y_{exp} - Y_{est})^2]$$

The loss function, or RMS was estimated as follow:

$$\text{Loss Function} - \frac{\sum (Y_{exp} - Y_{est})^2}{\text{Number of data points} - \text{Number of parameters}}$$

where Y_{exp} and Y_{est} are the experimental and estimated water activity values, respectively.

Molecular Dynamics Computations compared with NMR Relaxation

Computations and the employed methodology were as reported previously [2-10].

The Protein Aggregation Model Applied to Corn Zeins

This protein aggregation model takes account the following aggregation and water entrapment scheme

$$n\,P + q\,W \underset{k_2^n}{\leftrightarrow} P_nW_q$$

$$m(P_nW_q) + rW \underset{k_2^n}{\leftrightarrow} (P_nW_q)_m\,W_r$$

where P is the hydrated monomeric protein, k_1^n and k_2^n are the apparent association constants, and n, q, m, and r represent the numbers of cooperatively associating protein molecules, e.g., the initial formation of a n-mer from n protein monomers, followed by the association of m n-mers at the higher protein concentrations. Only protein-associated water in excess of that of the monomer P is indicated in these equations. Figure 2.3 illustrates the scheme in which the observed decrease in water activity (Aw) with increasing protein concentration is attributed to the entrapment of water within protein aggregates.

According to this model, the observed water activity can be expressed as a linear combination of weighted water activity contributions from the different protein species present,

$$A_w = f(w) + A_{w1}f(P_nW_q) + (A_{w2} - A_{w1})f((P_nW_q)_mW_r)$$

where A_{w1} and A_{w2} are the contributions from the water activities due to protein species P_nW_q and $(P_nW_q)_mW_r$) respectively, and $f(P_nW_q)$ and $f((P_nW_q)_mW_r)$ are the fractions of water bound and trapped by the corresponding protein species.

Through a mathematical re-arrangement, the two step aggregation model can be expressed as follows,

$$A_w = \frac{1}{1+K_1^nC^n} + A_{w1}\frac{K_1^nC^n}{1+K_1^nC^n} + (A_{w2}-A_{w1})\frac{K_2^mC^m}{1+K_2^mC^m} \qquad (2)$$

where the K's here are the equilibrium binding constant parameters, and n, m are the degrees of aggregation for apparent cooperative protein aggregation. C is the total protein concentration expressed in grams of protein/gram of water (which is converted from experimental moisture content data).

Results and Discussion

Water Sorption Isotherms

Protein water sorption phenomenon have been extensively studied. Since zeins are proteins, it is expected that the zein film should display behavior similar to proteins. Figure 1 shows the water sorption isotherms of zein powder, zein films without plasticizer, and glycerol plasticized zein films. It is clear from the figure that all the curves are sigmodial type. Due to the hydrophobic nature of corn zeins, water sorption is extremely low at low water activity range. Beyond this low range, water sorption increases exponentially with water activity. For zein powder and films without plasticizer, the water sorption is minimal when water activity is below about 0.8, while for glycerol plasticized film the value is about 0.7. Comparing zein powder with the unplasticized zein films, no significant difference in water sorption isotherms is observed. Both powder and films exhibit relatively low water content at high water activity (less than 50g/g of dry matter up to water activity of near 1.00). This suggests that the ethanol treatment during zein film formation has minimal influence on the water sorption capacity of the zein films.

A significant difference between the glycerol plasticized zein films and the unplasticized films is that the plasticized films display much higher water sorption capability at high water activity. When water activity is close to 1.00, water content of glycerol plasticized films is almost five times higher than that of unplasticized films. The higher water-holding capacity is contributed by the high hydrophilicity of glycerol. Glycerol has an overwhelming moisture affinity, resulting in an enhancement in sorption capacity for the plasticized zein films.

The results shown in Figure 1 are consistent with those obtained by Kanig and Goodman (1962). It was reported that RH of 10-52%, the moisture absorption was 1.5% or less, while at high RH such as 93%, film softened considerably and in some instances became completely deformed. Deformation and swelling of films occurring at high water activity range, as is also observed in the current study, cause significant structure and appearance changes of films, which in turn greatly affect both barrier and mechanical properties. The influence of sorption behavior on the characteristics of hydrophilic films such as zein and wheat protein-based films has been investigated extensively. Due to the importance of sorption behavior of films it is vital to accurately interpret this phenomenon.

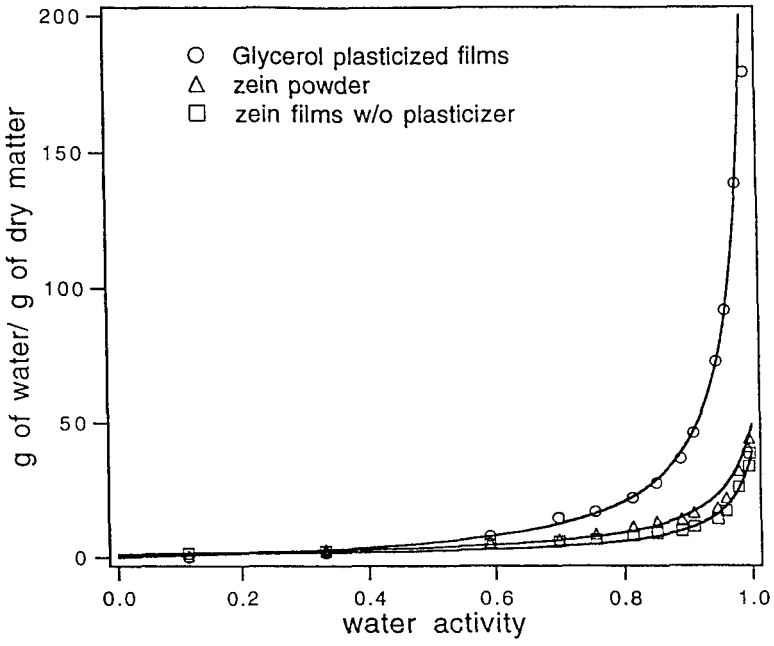

Figure 1. Water sorption isotherms of zein powder, zein films without plasticizer, and glycerol plasticized zein films at 20°C

Comparison of water sorption behaviors of various plasticized zein films are shown in Figure 2. They are glycerol, lactic acid, and polyethylene glycol plasticized zein films. All of them have similar sorption behaviors, with glycerol having the highest moisture affinity followed by polyethylene glycol and lactic acid. This difference is consistent with the hydrophobicity of these three plasticizers. Glycerol has the highest polarity in regarding to the number of hydroxyl groups. For the same plasticizer concentration (25% by weight) used in zein film formations, glycerol has the highest molar concentration. Thus, the total number of hydroxyl groups for glycerol is much higher than those for the other two and therefore, glycerol plasticized films exhibit the greatest water sorption capability.

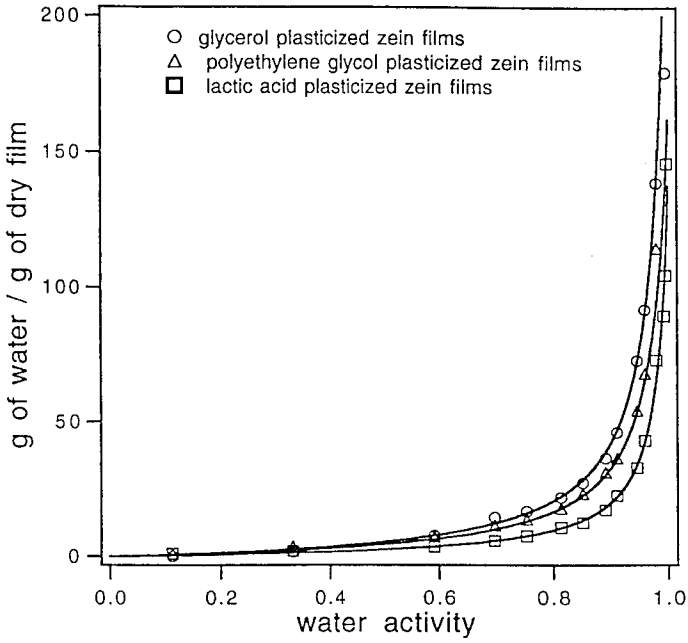

Figure 2. Water sorption isotherms of glycerol, lactic acid and polyethylene glycol plasticized zein films at 20°C

Analysis of Water Sorption Isotherms with a Protein Aggregation Model

In order to interpret the water sorption isotherm of films, a protein aggregation model was used. In this investigation, equation 2 will be used for the analysis of the data.

Results of nonlinear regression analysis are listed in Table 1. K_1 and K_2 denote the average equilibrium constants for the association of protein monomers that yield an oligomer during the corresponding cooperative aggregation steps. $\underline{n}$ and $\underline{m}$ represent the numbers of cooperatively associating protein molecules: initial formation of a n-mer from n protein monomers, followed by association of m n-mers at higher total protein concentration. The $\underline{n}$ and $\underline{m}$ terms reflect only those protein aggregation processes that are linked to water activity changes. A_1 and A_2 values are related to the water activity contributions of the different protein species.

Table 1. Results of nonlinear regression analysis of water sorption isotherms

	K_1	K_2	A_1	A_2	n	m	Loss Function
Zein powder	0.035±0.002	0.121±0.009	0.423±0.032	-0.306±0.029	4	4	0.002
Zein Film w/o plasticizer	0.024±0.002	0.080±0.005	0.364±0.038	-0.343±0.042	3	4	0.003
Zein Lactic Acid Film	0.020±0.001	0.107±0.008	0.460±0.025	-0.379±0.022	4	2	0.002
Zein Glycerol Film	0.032±0.001	0.196±0.013	0.562±0.027	-0.450±0.031	4	2	0.002
Zein PEG Film	0.040±0.0002	0.177±0.0009	0.526±0.025	-0.413±0.023	4	2	0.002
Native Wheat Gluten Powder	0.052±0.005	0.129±0.010	0.520±0.052	-0.393±0.059	4	4	0.003
Wheat Gluten Glycerol Film	0.037±0.013	0.192±0.046	0.559±0.105	-0.451±0.121	2	2	0.003

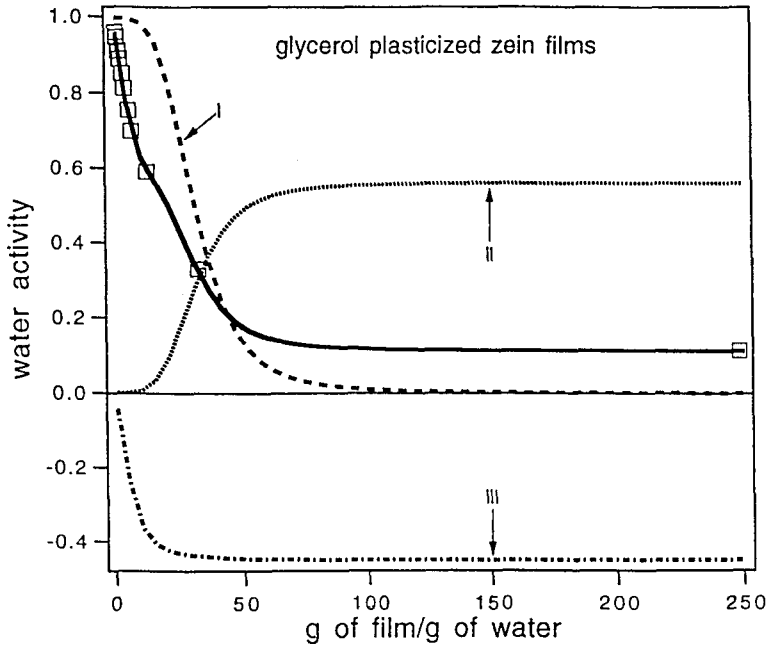

Figure 3. Nonlinear regression analysis of glycerol plasticized zein films

An example of experimental data and results from nonlinear regression analysis in the form of water activity versus film concentration is shown in Figure 3 for glycerol plasticized zein films. It should be noted that the data presented in the figures was converted from water sorption isotherm. The square symbols denote experimental data and solid lines are results of regression fitting. Different dashed lines (I, II, and III) represent water activity contributions of free (bulk) water, water entrapped in first and second type of aggregates respectively, and I, II, and III are corresponding to the terms in equation 2. As a general observation, it can be found that the free water contribution (Term I) decreased with increasing film or protein concentration. As film or protein concentration increases, the water activity contributed by Term I approaches zero. The contributions from Term II and III reach a plateau at concentrations higher than 100g film/g water. There are basically three protein or film concentration regions. When the protein or film concentration is low, water activity contributed by water entrapped in

first and second aggregates is minimal and free water is dominant in determining the water activity. When the film or protein concentration is high, contribution from each term stabilizes, resulting in low water activity. In the intermediate protein or film concentration range, the three terms influence the water activity almost equally.

A comparison of the results shown in Figure 4 indicates that there is no large difference between the zein powder and the unplasticized zein films. However, at high protein or film concentration range, zein powder exhibits higher water activity than the zein film. For example, when the protein concentration is 150g protein/g water, water activity of zein powder has the value of 0.1 while at the same protein concentration the water activity is minimal for zein films. At high protein concentrations (or low water content), water is either bound to polar groups of proteins or entrapped inside protein aggregates. As discussed in the previous section, the ethanol treatment has minimal effect on the water sorption isotherms of zein films. Therefore, the higher water activity at high protein concentrations might be contributed by looser structure of zein powder protein aggregates. The more compact structure of zein films may be due to the enhancement of the α-helix content of zeins by ethanol treatment during film formation.

Comparing the values of parameters in Table 1, one finds that K_1 and K_2 of the films are lower than those of zein powder. According to the protein aggregation model, K_1 and K_2 are the average apparent equilibrium constants for protein aggregate formation of Type I and Type II. Therefore, K_1 is affected primarily by non-covalent bonds (including H-bonds and electrostatic interactions), whereas K_2 values reflect mainly the protein aggregation through hydrophobic interaction. The lower K_1 value for the film in

Conclusions

1H NMR results of various corn zein films show that plasticizer molecular motions increase with water activity. Water has a plasticizing effect that leads to high degrees of motion of the components within the film matrix. Lactic acid plasticized corn zein films have lower water vapor permeabilities in comparison with glycerol and polyethylene glycol plasticized zein films. Nonlinear regression analysis of water sorption isotherm data for corn zein films using a protein aggregation model predicts that lactic acid plasticized zein films have the most compact film structure. This results is consistent with the predictions of our protein aggregation model.

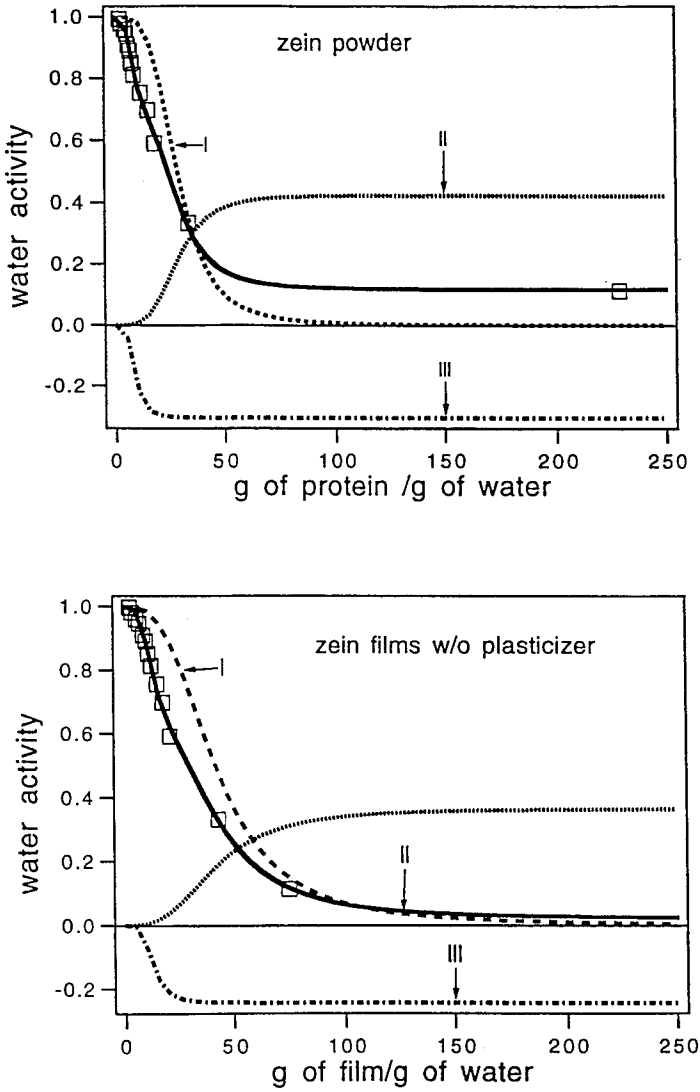

Figure 4. Nonlinear regression analysis of zein powder and zein films without plasticizer

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