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## Water Vapor Permeability and Solubility of Zein/Starch Hydrophilic Films Prepared from Dry Milled Corn Extract

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(Received 10 December 1995; revised 9 January 1997; accepted 10 January 1997)

### ABSTRACT

*Zein films containing different levels of starch were prepared from extracts of dry milled corn which had been previously rinsed in a hydrocyclone to improve the extractability of the zein as well as film formation. Zein was isolated from the rinsed dry milled corn by extraction with 70% (v/v) ethanol. Comparison of RP-HPLC profiles indicated the presence of  $\beta$ - and  $\gamma$ -zeins in the extracted isolate which were not found in commercial zein. Water vapor barrier properties of the glycerine-plasticized films prepared from extracted zein isolates, containing approximately 8% starch, were comparable to films prepared from commercial zein. Water resistance of zein isolate films decreased with increasing starch content. © 1997 Elsevier Science Limited*

### INTRODUCTION

Ethyl alcohol produced by fermentation of corn raw materials is an important and growing constituent of renewable liquid fuel in the United States. In order to support expansion of the corn ethanol process, profitable markets must be identified for new non-starch coproducts derived from corn. Edible films and coatings have been prepared from grain proteins such as soy, wheat and corn (Gennadios and Weller, 1990). Wheat gluten and corn zein are alcohol soluble proteins that exhibit hydrophobic properties. Zein proteins form characteristically hard and glossy coatings on pharmaceutical tablets and candies (Gennadios and Weller, 1990). Park *et al.* (1994) have coated tomatoes with corn zein films of different thickness to optimize the O<sub>2</sub> content of the film in order to improve their storage life and quality. Grease resistance of corn zein coated paper has been measured, as part of a program to develop alternative packaging material to polyolefin wrapping materials used by the fast food industry (Trezza and Vergano, 1994).

Commercially, zein has been isolated from corn gluten meal (60% protein) using a two-step extraction process consisting of hot aqueous isopropyl alcohol and hexane (Pomes, 1971). A one-step process is more commonly employed to extract zein from corn gluten at 60°C using 88% (v/v) isopropanol containing 0.25 wt% NaOH (Reiners *et al.*, 1973): Less than half of the protein in the meal, however, is isolated at low temperatures after the supernate is decanted. More recently, ethanol 70% (v/v) has been used for quantitative extraction of zeins from the endosperm of corn kernels (Wilson, 1991; Dombink-Kurtzman, 1994). In addition Tsai (1980), demonstrated that at least 1 mM of 2-mercaptoethanol in the alcohol prevented protein aggregation and maximized zein extraction. Coupled with RP-HPLC, the maize alcohol-soluble proteins can be separated into four groups and identified. Some of the minor high-sulfur proteins present in laboratory zein preparations (Wilson, 1986) are not found in commercial zein preparations. These  $\beta$ - and  $\gamma$ -subunits present in laboratory preparations reportedly contribute to improved antioxidant activity of zein against docosahexaenoic acid ethyl ester (Matsumura *et al.*, 1994).

In this study, zein films, containing different levels of starch, were prepared from extracts of corn previously ground and rinsed using procedures deemed to be practical and economical at large scales. The films were targeted as packaging material for foods which require controlled water vapor transport to extend the shelf-life of the product. Solubility and water vapor barrier properties of the zein/starch films made from the extracted material are reported.

## EXPERIMENTAL PROCEDURES

### Materials

Corn, yellow dent from Davis Feeds (Perkasie, PA, USA) and corn zein (F-4000) protein obtained from Freeman Industries (Tuckahoe, NY, USA) were used. Glycerine 85% (J.T. Baker Chemical Co., Phillipsburg, NJ, USA) was used as the plasticizer in the films.

### Grinding

Table 1 describes two methods used to grind the corn. In a two stage method the corn was ground on a #1 Wiley mill (Arthur H. Thomas Co., Philadelphia, PA,

**TABLE 1**  
Corn Milling and Rinsing

<i>Corn treatment</i>	<i>Batch 2</i>	<i>Batch 3</i>	<i>Batch 30</i>
Milling	2 stages	2 stages	1 stage
Initial 8% NaCl rinse	no	no	yes
Corn (kg)	3.2	6.8	25.2
Time (h)	1.0	4.2	2.8
Mass ratio water/corn	16.6	21.3	19.2

USA) in the first stage, reducing the whole kernels to <3 mm using a 3 mm screen at 1.4 kg/min. A second grinding with a 1 mm screen in the mill, at 6.8 kg/h, reduced the particle size to <1 mm. We found that corn could be reduced from whole kernels to <1 mm, using the 1 mm screen, as rapidly as regrinding the <3 mm corn powder, this one-stage method was used thereafter.

### **Corn powder rinsing**

Two similar rinsing procedures were used to rinse the corn ground in two stages (Table 1). In a run called Batch 2 in this report, 3.2 kg of corn powder ground 3 months earlier, was added to 15.1 kg of tap water in a tank and mixed by pumping through a small centrifugal pump (model c114C, Alfa Laval, Kenosh, WI, USA) for an hour. The corn powder slurry was then pumped through a small hydrocyclone at 1050 kg/h. The hydrocyclone was built for an earlier project and has a 22 cm conical section, 5 cm at the top and 1.5 cm at the bottom outlet. The (lighter) slurry fraction pumped out of the top of the hydrocyclone was discarded and the bottom stream returned to the tank. When the tank was drained down to minimum volume for recirculation, 7.5 kg of tap water were added. Four more 7.5 kg batches of water were added, so that, in all, 53 kg of water was used for a total hydrocyclone separation time of one hour. The number of rinsing batches was selected as that sufficient to reduce the turbidity of the overflow to that of water. The powder was drained and evaporated at room temperature overnight to 27% moisture on a dry basis. The powder was then freeze dried (Labconco freeze dryers, Labconco Corp, Kansas City, MO, USA) for storage before zein extraction.

In a run called Batch 3, 68 kg of corn powder, ground the previous day, was added to 320 kg of tap water in a 680 litre kettle. This mixture was agitated with a propeller blade mixer for one hour without recirculation through the hydrocyclone. Then it was recirculated through the hydrocyclone as before. Corn slurry from the bottom outlet was returned to the top tank. The effluent from the upper hydrocyclone outlet was discarded and the tank drained for 50 min down to about 77 kg of slurry. The draining stop point was visible evidence of the larger corn particles in the upper effluent stream. Thirty gallons of tap water was added to the kettle, taking 4.5 min, and the light fraction of the diluted slurry was again drained from the upper outlet of the hydrocyclone for 17 min. Nine more 13.6 kg rinses were made taking 205 min from the beginning of the first 30 gallon water rinse to the completion of the last one. The heavy fraction of the corn powder was drained through a screen, spread in a single-particle thick layer and dried overnight. The following day the rinsed corn powder was dried under a vacuum of 100  $\mu\text{m}$  Hg (13 Pa) for 12 h at  $25 \pm 2^\circ\text{C}$  to 4.7% moisture.

In a run called batch 30, 25.2 kg of ground corn was mixed with 112 kg of 8% NaCl solution at  $19.4^\circ\text{C}$  for one hour, then pumped through the hydrocyclone and the top effluent was drained at 270 kg/h for 22 min. While continuously draining, 38 kg dilutions of water were added at 8–10 min intervals. The rinsed, extracted corn was finally drained into buckets after an overall mixing process of 170 min, with a final solution salt content of 0.03% by conductivity. After drying at  $25 \pm 2^\circ\text{C}$  for 2 days to 5% moisture, 2.6 kg (10.3% of original powder mass) was recovered. Seven washes were sufficient to reduce the salt content to the final level.

### **Extraction of zein from rinsed corn**

Twenty grams of rinsed or unrinsed corn powder was extracted with 200 ml 70% (v/v) ethanol for 2 h at 60°C. Insolubles were removed by centrifuging at 1000 × g for 15 min at 23°C. Ethanol was removed from the extract by evaporation. Typically, the average yield of extracted zein for five corn powder replicates was 3.88 ± 0.28%.

### **Chemical analysis**

Protein content for milled corn and zein isolates was obtained using the Kjeldahl method given in AOAC 2.055 (AOAC, 1984) using a factor of 6.38. Starch content was determined according to a previously published procedure (McCready *et al.*, 1974) by measuring the amount of glucose present in trifluoroacetic acid (TFA) hydrolyzed samples using HPLC and an Aminex HPX-87H column (Bio Rad, Hercules, CA, USA). Oil content was determined by packing a glass-wool plugged pipette with approximately 100–300 mg of sample, previously dried at 110°C overnight. The micro column was eluted with 5 ml of hexane followed by 5 ml of chloroform. The eluates were collected in a tared vial and subsequently evaporated to a constant weight with a stream of nitrogen gas and the weights of hexane and chloroform extracts determined.

### **Chromatography**

The RP-HPLC method used to separate the corn protein has been described elsewhere (Wilson, 1991; Dombink-Kurtzman, 1994). Commercial and isolated zein samples were defatted as described earlier and 24 mg of defatted zein was dissolved in 2 ml 70% (v/v) ethanol, 5% (v/v) 2-mercaptoethanol (2-ME), and 0.5% (w/v) sodium acetate, then vortexed at room temperature. Insolubles were removed by centrifuging at 12000 × g for 5 min at room temperature in an Eppendorf centrifuge and the supernatant was filtered through a 0.45 μm filter. The extracted proteins were diluted 1:10 with 55% (v/v) isopropanol, 5% (v/v) 2-ME and separated on a Vydac (Hesperia, CA, USA) RP-C<sub>18</sub> 5μ, 300 Å analytical column (25 cm × 4.6 mm) which was protected with a Vydac RP-C<sub>18</sub> guard column. Zein proteins were separated using a 0.1% trifluoroacetic acid–acetonitrile (TFA–ACN) gradient at 1.0 ml/min, 55°C, and monitored at 210 nm. The gradient started at 38.3% ACN, increasing at 0.502%/min for 13.6 min; at 1.95%/min for 2.8 min; at 0.100%/min for 13 min; at 0.1986%/min for 18 min and at 2.48%/min for 3.8 min; ending at 64.92%.

### **Film formation**

Film forming solutions were prepared using a modification of the procedure of Gennadios and Weller (1994). Commercial or isolated zein, 0.7 g and 0.3 g of glycerine were dissolved in 30 ml 95% (v/v) ethanol with gradual heating to 70°C over 10 min. While still hot, 10 ml of water was added (final ethanol concentration 70% v/v) and the clear solution was poured into a disposable polystyrene petri dish (100 × 15 mm). The solvent was removed from the film by placing the dish in an air-circulating oven (Lab-Line Instruments, Inc., Model 3471M, Melrose Park, IL,

USA) maintained at 35°C for 15 h, which were then stored in an incubator at  $25 \pm 2^\circ\text{C}$  and RH  $50 \pm 5\%$  for approximately 24 h before testing.

#### **Film thickness measurement**

A micrometer (Tumico, St. James, MN, USA) was used to measure film thickness. Reported thickness was the mean value of ten random measurements made before and after testing.

#### **Water vapor permeability (WVP) determination**

The apparatus and methodology described in the ASTM E96-80 (ASTM, 1980) 'Water Method', as modified by McHugh *et al.* (1993), was used to measure the WVP of the films. Four film replicates of each batch were prepared as described earlier and tested at  $30 \pm 2^\circ\text{C}$ . Cast films were smooth on the side facing the petri dish surface. Films were sealed on Plexiglas cups containing 9 ml distilled water with the smooth side positioned toward the water. There was an air gap of 0.6 cm between the water and the underside of the film. Test cups were placed in a desiccator cabinet maintained at 0% RH with calcium sulfate. A Boxer Fan, model W52107F, IMC Magnetic Corp. (Tempe, AZ) was used to develop air velocities of approximately 150 m/min across the films. Air velocities were measured with a ThermoAnemometer (model 8565, Alnor Instrument Co., Skokie, IL, USA). Cups were weighed a minimum of five times at time intervals no less than 3 h apart. The water vapor permeability was calculated from a linear regression of the slope of weight loss vs time. Water vapor transmission rate (WVTR) was calculated by dividing the slope by the test cell mouth area. Permeance  $[\text{WVTR}/(p_2 - p_3)]$  was calculated at  $30^\circ\text{C}$  where  $p_2$  and  $p_3$  are the corrected partial pressures at the inner surface of the film and at the film outer surface, respectively (McHugh *et al.*, 1993). Water vapor permeability was calculated as the product of the permeance and average thickness of the film.

#### **Solubility in water**

The method of Gontard *et al.* (1992) was used to determine the percentage of film solubilized after 24 h immersion in water at  $23 \pm 2^\circ\text{C}$ . Weighed 3 cm film discs, dried at  $100^\circ\text{C}$  for 24 h, were placed in a petri dish containing 50 ml of water and 0.02% w/v sodium azide. The solution was stirred for 24 h at  $23 \pm 2^\circ\text{C}$ , the liquid poured off and then dried at  $100^\circ\text{C}$  for 24 h. Solubility in water was calculated by subtracting the weight of dry matter remaining from the weight of initial dry matter and reported on initial dry weight basis.

## **RESULTS AND DISCUSSION**

Corn was ground on a Wiley mill to specific mesh size and subjected to different water rinsing procedures to remove various amounts of starch, protein and oil which were expected to improve the extractability of the corn zeins and improve film

**TABLE 2**  
Analysis of Dry Milled Corn

Sample	Protein %	Starch %	Protein/ starch	Oil %			Moisture %
				Hexane	Chloroform	Total	
Corn powder	8.47	46.7	0.18	3.37	0.50	3.87	13.3
Batch 2	8.73	71.4	0.12	0.29	0.17	0.46	4.7
Batch 3	9.68	71.9	0.13	0.52	0.01	0.53	3.7
Batch 30	8.50	64.0	0.13	0.35	0.47	0.82	8.8

formation. Dry milled corn samples were analyzed for protein, starch and oil. Analysis of rinsed corn powder showed it contained typical amounts of protein, but the amount of starch varied with rinse treatment (Table 2). Batch 3, which was rinsed with roughly the same proportion of water to corn as Batch 2, but with the powder in much longer contact with the water compared to Batch 2, contained more protein. However, the time per unit mass of corn was much less, which indicates that the contact time was sufficient to dissolve soluble constituents from the corn powder and composition variation is probably due to an insufficient ratio of water/corn powder. Batch 30 was initially mixed with an 8% NaCl solution, which was expected to provide greater buoyancy to germ particles and possibly improve oil separation, contained less starch and protein than either Batch 2 or 3. Apparently most of the oil rich particles were removed during separation of the lighter particles in hydrocyclone by either water or saline rinses.

Zein isolated by extraction with 70% (v/v) ethanol at 60°C from the rinsed, dry milled corn contained nearly twice the weight fraction of protein found in the corn powder (Table 3). In general, isolates from rinsed corn powder contained protein levels comparable to commercial zein. The protein content of the isolate from batch 3 was almost 90% and comparable to commercial zein which is typically obtained by extraction from corn gluten. Zein isolates also contained more starch and extractable oil than commercial zein. The isolate from batch 2 contained more starch than the other rinsed samples and the isolate from batch 30 contained the most extract-

**TABLE 3**  
Analysis of Zein Isolate

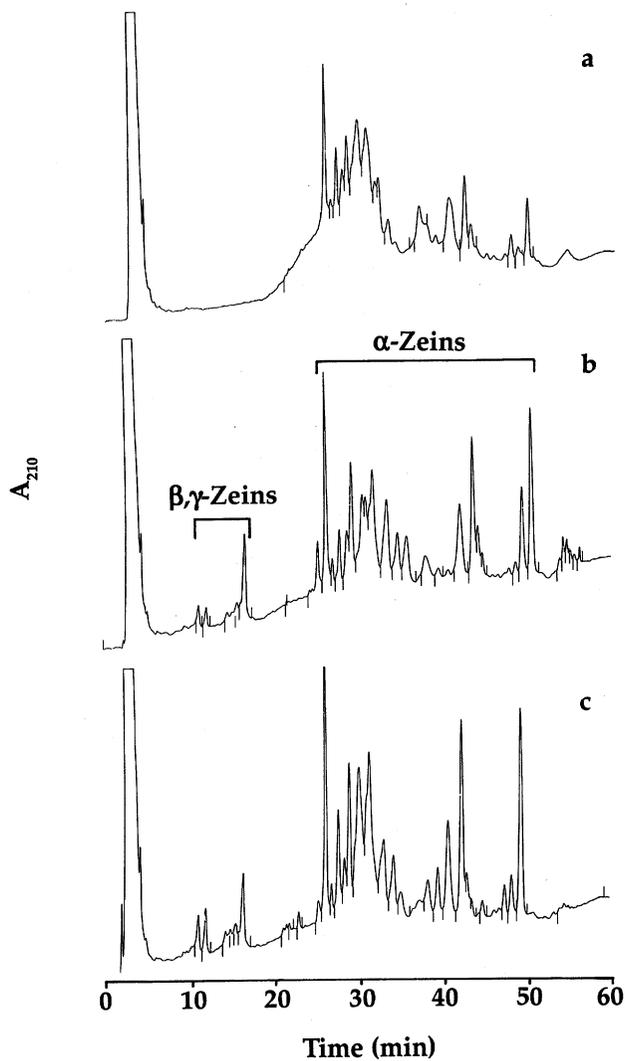
Sample	Protein %	Starch %	Oil %		Moisture %
			Hexane	Chloroform	
Corn powder <sup>a</sup>	48.23	13.6	2.15	5.76	6.2
Batch 2 <sup>a</sup>	77.08	7.8	2.94	1.85	5.8
Batch 3 <sup>a</sup>	88.39	1.1	1.87	1.38	5.1
Batch 30 <sup>a</sup>	85.90	2.0	5.35	1.78	5.0
Commercial zein <sup>b</sup>	90.51	0.1	1.07	0.00	5.2

<sup>a</sup>Zein was extracted from dry milled corn with 70% (v/v) ethanol at 60°C for 2 h.

<sup>b</sup>Freeman F-4000.

able oil. Losses associated with these analyses can be attributed to incomplete extraction of various components in dry milled corn or zein isolate (Tables 2 and 3). No attempt was made to analyze for cellulosic material in the corn.

Alcohol-extractable corn proteins in commercial zein and the zein isolates was further characterized by RP-HPLC (Fig. 1). Comparisons of their chromatographic profiles indicated that the isolates contained a greater number of resolved peaks



**Fig. 1.** RP-HPLC separation of alcohol soluble zein proteins. (a) commercial zein (Freeman F-4000). (b) Isolate extracted from dry milled corn Batch 2. (c) Isolate extracted from dry milled corn Batch 3.

compared to the commercial product. The broader, poorly resolved peaks displayed in the profile for commercial zein (Fig. 1a) could be attributed to deamidation reactions which can result from extraction under alkaline conditions (Reiners *et al.*, 1973). Early eluting peaks (10–17 min), which are present in the chromatographic profiles for the zein isolates (Fig. 1b and c), represent  $\beta$ - and  $\gamma$ -zein, and are not found in commercial zein (Fig. 1a). These subunits contain more sulfur amino acids, such as cysteine and methionine, than the  $\alpha$ -subunits (Pederson *et al.*, 1986). The more hydrophobic  $\alpha$ -zeins comprise the major storage protein fraction and elute later, between 25 and 50 min.

Glycerine-plasticized zein films were prepared from the isolates and commercial zein to determine the effect of starch and oil on the film's solubility and barrier properties. Zein isolated from unrinsed corn powder did not form a film. This could be attributed to the low protein content of the isolate or the presence of soluble proteins and cellulosic material removed during rinsing. The water vapor barrier properties of the film-forming isolates was found to be better for films containing less starch (Table 4). Water vapor permeability (WVP) values were lower for films prepared from Batch 3 isolate (1% starch) than isolates from Batches 2 or 30, which contained approximately 8% and 2% starch, respectively. WVP values for the latter two isolates were comparable to those prepared with commercial zein which contained essentially no starch. Even though prolamines are alcohol soluble proteins, zein films are considered hydrophilic. The relative humidity (RH) values, on the underside of the film during WVP testing, ranged between 84.6 and 91.4%. This indicates that water vapor is readily transported across the film surface. Another important property of hydrophilic films is their water resistance, particularly where water activity is high or when the film is in contact with aqueous solutions for prolonged periods of time. Zein films have good water resistance properties compared to other edible protein films such as wheat gluten (Gontard *et al.*, 1992). The solubility of the films prepared from zein isolates increased with increasing starch content (compare Tables 3 and 4). However, commercial zein films, which contained essentially no starch, were the most water soluble of the films tested. It appears

**TABLE 4**  
Water Vapor Permeability and Solubility of Zein/Starch Films

<i>Film<sup>a</sup></i>	<i>Thickness (mm)</i>	<i>RH (%)<sup>b</sup></i>	<i>WVP (g-mm/kPa-h-m<sup>2</sup>)<sup>c,d</sup></i>	<i>Material Dissolved in Water %<sup>d</sup></i>
Batch 2	0.0800	88.4	1.21a ± 0.16	15.73b ± 0.11
Batch 3	0.0841	91.4	0.91b ± 0.06	14.18c ± 0.12
Batch 30	0.0564	84.6	1.18a ± 0.03	14.38c ± 0.24
Commercial zein <sup>e</sup>	0.0930	89.9	1.21a ± 0.15	16.90a ± 0.98

<sup>a</sup>Films were prepared in 70% (v/v) ethanol.

<sup>b</sup>Relative humidity at the inner surface of the film.

<sup>c</sup>WVP values corrected for stagnant air effects, at 30°C.

<sup>d</sup>Means with no letter in common are statistically significant ( $p < 0.05$ ) by use of Bonferroni lsd multiple-comparison method.

<sup>e</sup>Freeman F-4000.

therefore that the dissolved material did not contain a significant amount of soluble starch, but mostly glycerine.

In conclusion, hydrophilic films prepared from zein isolates, containing 1% starch, exhibited lower WVP values and were more water resistant than films prepared from commercial zein. Up to 8% starch, extracted with zein from dry milled corn, was incorporated into glycerine-plasticized films with no significant loss to water vapor permeability and improved water resistant properties. Future research will be directed toward the development of novel and more economical extraction techniques of corn zeins for the preparation of biopolymer films and coatings with desired strength and water vapor properties to extend the shelf-life of foods and other water sensitive materials.

## REFERENCES

- ASTM (1980). Standard test method for water vapor transmission of materials. E96-80. In *Annual Book of ASTM*, pp. 771–778. ASTM, Philadelphia, PA, USA.
- Dombrink-Kurtzman, M. A. (1994). *Journal of Cereal Science*, **195**, 57–64.
- Gennadios, A. & Weller, C. L. (1990). *Food Technology*, **44**, 63–69.
- Gennadios, A. & Weller, C. L. (1994). *Transactions of the ASAE*, **37**, 535.
- Gontard, N., Guilbert, S. & Cuq, J. (1992). *Journal of Food Science*, **57**, 190–199.
- Matsumura, Y., Andonova, P. P., Hayashi, Y., Murakami, H. & Mori, T. (1994). *Cereal Chemistry*, **71**, 428–433.
- McCready, R. M., Ducay, E. D. & Gauger, M. A. (1974). *Journal of the AOAC*, **57**, 336–340.
- McHugh, T. H., Avena-Bustillos, R. & Krochta, J. M. (1993). *Journal of Food Science*, **58**, 899–903.
- AOAC (1984) Official Methods of Analysis of the Official Analytical Chemists, ed. S. Williams, 14th edn. AOAC Publisher, Arlington, VA, USA.
- Park, H. J., Chinnan, M. S. & Shewfelt, R. L. (1994). *Journal of Food Science*, **59**, 568–570.
- Pederson, K., Argos, P., Naravana, S. L. & Larkins, B. A. (1986). *Journal of Biological Chemistry*, **261**, 6279–6284.
- Pomes, A. F. (1971) In *Encyclopedia of Polymer Science and Technology: Plastic, Resins, Rubbers, Fibers*, eds H. F. Mark, N. G. Gaylord and N. M. Bikales, Vol. 15, pp. 125–132. Interscience Publishers, NY, USA.
- Reiners, R. A., Wall, J. S. and Inglett, G. E. (1973) In *Industrial Uses of Cereals*, ed. Y. Pomeranz, pp. 285–302. American Association of Cereal Chemists, Inc., St. Paul, MN, USA.
- Trezza, T. A. & Vergano, P. J. J. (1994). *Food Science*, **59**, 912–915.
- Tsai, C. Y. (1980). *Cereal Chemistry*, **57**, 288–290.
- Wilson, C. M. (1986). *Plant Physiology*, **82**, 196–202.
- Wilson, C. M. (1991). *Plant Physiology*, **95**, 777–786.