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Effect of Endogenous Triacylglycerol Hydrolysates on the Mechanical Properties of Zein Films from Ground Corn

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Dry-milled yellow corn and freshly ground food and nonfood grade yellow and white hybrid corn kernels were pretreated in a solution of lactic acid and sodium metabisulfite followed by extraction with 70% ethanol. Zein was precipitated from the extract by reducing the ethanol content of the extract to 40%. Lipid associated with the zein isolates was between 15 and 20% and contained mostly endogenous free fatty acids. The effect of the endogenous free fatty acids on zein isolate films, with and without free fatty acids, was determined by measuring various film properties. Stress−strain measurements indicated 40−200% greater elongation for zein films containing endogenous free fatty acids. Films prepared from zein isolated from preground corn stored for ∼4 months (27 °C, 17% relative humidity) had ∼3 times greater elongation values than zein films prepared from freshly ground corn.

KEYWORDS: Corn; hybrid; dry-milled; zein; endogenous; fatty acids; films; mechanical properties

INTRODUCTION

Zeins, the maize storage proteins, are the most abundant proteins in the corn endosperm and account for more than half the total mass of the seed protein (*1*, *2*). Zein is extracted commercially with aqueous alcohol from corn gluten meal after most of the oil has been removed. The film-forming properties of zein have long been recognized (*1*). However, films prepared from commercial zein are generally too brittle and exhibit tensile strength too low for most applications. Elongation values of zein films increase significantly when plasticized with polyethylene glycol 400 (*3*). Zein films plasticized with glycerol/polypropylene glycol (1:3 weight ratio) had elongation values almost 50 times greater than those of glycerol-plasticized films (*4*). The properties and microstructure of cast and resin zein films plasticized with oleic acid (*5*) and zein resin films plasticized with palmitic and stearic acids (*6*) have been reported. Handa et al. (*7*) observed an increase in tensile strength and elongation values for egg white/oleic acid films, which they attributed to unfolding of the egg white protein chains through association with oleic acid. Forato et al. (*8*) demonstrated the presence of free fatty acids within both the protein bodies of the corn endosperm and also the alcohol-extracted α-zeins. It was also shown that the presence of free fatty acids in zein isolated from dry-milled corn enhanced the grease resistance and water vapor barrier properties of paper coated with the isolate (*9*). In this study, the effect of endogenous free fatty acids on the mechanical properties of films prepared from zein isolated from dry-milled, bulk yellow dent, and ground hybrid corn was investigated.

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MATERIALS AND METHODS

Materials. Corn, yellow dent, was milled to a particle size of 20 mesh with a counter-rotating ribbed disk mill at Davis Feeds, Perkasie, PA, and stored at 4 °C. Controlled hybrid corn was generously supplied by Pioneer Hi-Bred International, Inc., Johnston, IA. Yellow hybrid corn 34K77 is of food grade and 33A14 is not of food grade but is high in extractable starch. White corn 32H39 is a leading white hybrid planted over 50% of the total United States white corn acreage in 1999, and white hybrid 32K72 was first planted commercially in 1999. Hybrid corn was ground, in the laboratory, to a particle size of 20 mesh with a Wiley mill, from Thomas Scientific, Philadelphia, PA, and stored at 4 °C. Zein, F-4000, was obtained from Freeman Industries, Tuckahoe, NY. Lactic acid 85% (w/w) and sodium metabisulfite (ACS reagent) were obtained from Sigma, St. Louis, MO, and poly(ethylene glycol), average MW ∼400 (PEG), and linoleic, oleic, and palmitic acid were from Aldrich Chemical Co., Inc., Milwaukee, WI.

Zein Isolates. Treatment of dry-milled corn (DMC) before extraction significantly reduced the formation of insoluble protein aggregates in zein isolates (*10*). Therefore, a mixture of 100 g of DMC or ground hybrid corn (GHC) was stirred at 50 °C in 500 mL of water containing 2.5 mL of lactic acid and 1.26 g of sodium metabisulfate for 6 h. The insoluble corn was collected by filtration, then mixed, and washed with 400 mL of water. The treated corn was then batch extracted with 500 mL of 70% ethanol at 60 °C for 2 h. Insolubles were separated from the extract by vacuum filtration and discarded. The extract was diluted to 40% ethanol with water and stored at 4 °C overnight. The precipitated zein was isolated from the extract by centrifugation at 10,400*g* and 4 °C for 15 min and lyophilized.

Chemical Analysis. Protein, starch, and total lipid content of DMC samples was determined as described earlier (*9*). Protein, starch, and lipid content of GHC samples was furnished by Pioneer Hi-Bred International. Nonpolar lipids in the zein isolates from DMC and GHC (2 g) were extracted with 40 mL of hexane in a 50 mL screw-top tube. The samples were inverted 30 times and then placed on a Burrell Wrist Action shaker at full speed for 1 h. Following shaking, the extraction

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Table 1. Proximate Composition of Corn Samples[a]

corn type	wt % protein	wt % starch	wt % lipid
DMC	8.5	71.4	3.9
YH33A14	8.1	72.6	3.5
YH34K77	9.1	71.2	4.2
WH32H39	8.9	72.0	4.0
WH32K72	8.9	71.8	4.2

[a] At 0% moisture.

solvent was separated from the solid sample by vacuum filtration with a Büchner funnel and Whatman GF/A filter paper. The extraction solvent was collected in a 50 mL tube, and the solid material was recovered from the filter paper.

High-Performance Liquid Chromatography (HPLC). Lipid in the hexane extracts from the zein isolates was separated and quantified according to the method of Moreau et al. (*11*). The separation was carried out on a LiChrosorb Diol column 5 mm (3 × 100 mm) using a ternary gradient system consisting of hexane/2-propanol/acetic acid at a constant flow rate of 0.5 mL/min. Eluting peaks were detected with an Alltech-Varex Mark III evaporative light scattering detector (ELSD) at 40 °C with nitrogen as the nebulizing gas at a flow rate of 1.6 L (STP)/min.

Film Formation. A total of 0.5 g of zein and plasticizer, in various proportions, was dissolved in 10 mL of 90% ethanol to yield a 5% (w/w) mixture. The mixture was heated with stirring at 70 °C for 10 min, then cast in polystyrene Petri dishes, and dried in a vacuum oven, adjusted to 10 in. of mercury at 50 °C, for ~4 h. The dried films were stored at 23 ± 1 °C for at least 24 h in a desiccator held at 52% relative humidity (RH) by means of a saturated solution of sodium hydrogen sulfate in a dish in the bottom of the desiccator and then evaluated.

Tensile Property Measurements. Tensile properties were determined using an Instron model 1122 tensile tester with a 2000 g load cell. Specimens were 5.0 mm wide and were in the range of 0.08−0.2 mm thick. Thickness was measured using a micrometer. For tensile measurements, a gauge length of 25 mm and extension rate of 5 mm/min were used. Five replicates were run for each material. Samples were conditioned overnight in a desiccator held at 52% relative humidity (RH) by means of a saturated solution of sodium hydrogen sulfate in a dish in the bottom of the desiccator. Individual specimens were stored in the desiccator until they were clamped into the Instron. The jaw clamps were operated using 45 psi of air pressure. Data were collected and analyzed for average standard deviation using the DOS-based Series IX, version 6, Instron software.

RESULTS AND DISCUSSION

The effect of endogenous free fatty acids on the mechanical properties of zein films was investigated for zein films prepared from dry-milled, yellow dent corn (DMC) and ground yellow hybrid corn (YHC) and white hybrid corn (WHC). The proximate composition range (dry basis) listed in **Table 1** for the different corn samples was narrow, with protein content between 8.1 and 9.1%, starch content between 71.2 and 72.6%, and lipid content between 3.5 and 4.2%. The protein content (dry basis) of the zein isolates was between 80 and 81% compared to 88−96% reported for commercial zein. The amount of nonpolar lipids in the hexane extract from zein isolates was between 15 and 20%. The principal lipid components detected by evaporative light scattering (ELSD) in hexane extracts were triacylglycerols (TAG) and free fatty acids (FFA). The amount of TAG in zein isolates has been shown to be significantly less than that found in milled corn (*9*). Most of the TAG (which comprise 99% of the lipids in the kernel) are removed with the insolubles after extraction of the zein from the ground corn. The lipid composition of zein isolate from DMC contained the highest concentration of FFA and the lowest concentration of TAG of the isolates examined (**Table 2**). The zein isolate from

Table 2. Lipid Composition of Hexane Extract from Zein Isolates

corn type	wt % lipid TAG	wt % lipid FFA	wt % lipid St	wt % lipid FPE
DMC	7.11	80.29	4.95	0.50
YH33A14	26.84	58.13	12.51	0.95
YH34K77	29.78	66.97	13.47	1.22
WH32H39	19.68	63.06	12.24	1.05
WH32K72	14.50	48.92	6.86	0.65

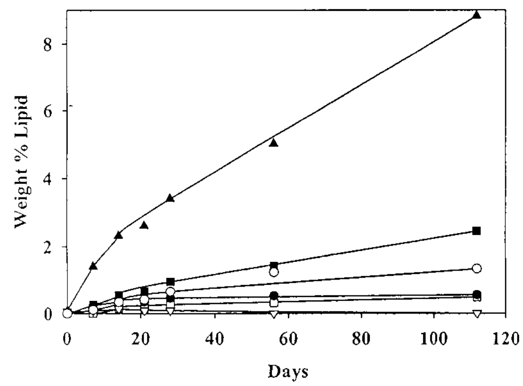

Figure 1. Hydrolysis of TAG in corn YH34K77 stored at 26.8 ± 0.2 °C and 17 ± 1% RH: (preground) ▲, linoleic acid; ■, oleic acid; ○, 1,3 DAG; ●, 1,2 DAG; □, palmitic acid; (freshly ground) ▽, linoleic acid.

YHC and WHC contained from 14.50−29.78% TAG and 48.92−66.97% FFA. Two other components in the lipid fraction of the isolate were phytosterols (St) and free phytosterol esters (FPE), which, apparently, were concentrated during the isolation procedure. The distribution of FFA in the zein isolates was linoleic > palmitic > oleic > stearic.

The high FFA and low TAG values in the zein isolate from DMC are probably due to hydrolysis of TAG during storage of the corn. To study the effect of storage conditions on TAG hydrolysis, corn YH34K77 was divided into two portions and stored under controlled conditions (26.8 ± 0.2 °C and 17 ± 1% RH). One portion was ground at the beginning of the study (preground) and the other was ground just before sampling (freshly ground). Over a 112 day period 16.4% of TAG were hydrolyzed to FFA and diacyglycerols (DAG) in the preground corn (**Figure 1**). Very little hydrolysis of TAG occurred in corn freshly ground before analysis. The amount of FFA found was linoleic > oleic > palmitic. Similarly, 1,2- and 1,3-DAG were present only in the hexane extracts of preground corn samples. More 1,3-DAG (1.35%) than 1,2-DAG (0.56%) was found in preground corn samples, which could be attributed to steric hindrance of lipase.

The effect of the endogenous lipids on the mechanical properties of clear cast films of the isolates was determined from stress−strain measurements of films with various lipid contents. The tensile strength (TS) and elongation to break (ETB) of the zein film were measured. Initial modulus, a measure of resistance to stretching, or the stiffness of the film was determined from the TS trace. Zein isolate films prepared from DMC were more flexible, less stiff, and much weaker than the films from the other zein isolates (**Table 3**). Elongation values for films from isolates of the four types of ground hybrid corn were statistically the same; however, TS and initial modulus values varied. Zein films from YH33A14 and WH32H39 were not as strong and less stiff than films from isolates YH34K77 and WH32K72 as indicated by their TS and initial modulus values. These observations could not be attributed to greater amounts of FFA in the isolates (**Table 2**). ETB values for all

Table 3. Mechanical Properties of Zein Isolate Films Containing Endogenous Fatty Acids[a]

corn type	TS (MPa)	ETB (%)	modulus (MPa)
DMC	5.3 (0.5)[c]	7.1 (1.7)[a]	123 (42)[c]
YH33A14	15.8 (1.8)[b]	3.0 (0.3)[b]	693 (77)[b]
YH34K77	24.0 (7.1)[a]	3.3 (0.8)[b]	1235 (334)[a]
WH32H39	13.6 (3.0)[b]	3.2 (0.3)[b]	567 (180)[b]
WH32K72	19.4 (3.6)[ab]	3.1 (0.3)[b]	1206 (208)[a]

[a] Values in parentheses indicate standard deviation. Within each category, means with no superscript in common are significantly different ($P < 0.05$)

Table 4. Mechanical Properties of Zein Isolate Films with Endogenous Fatty Acids Removed[a,b]

corn type	TS (MPa)	ETB (%)	modulus (MPa)
DMC	14.5 (2.1)[a]	2.4 (0.2)[a]	746 (90)[b]
YH33A14	13.0 (2.7)[ab]	1.4 (0.2)[b]	1057 (102)[ab]
YH34K77	11.6 (2.0)[ab]	1.3 (0.3)[b]	1108 (199)[ab]
WH32H39	15.8 (3.8)[a]	2.3 (0.3)[a]	829 (200)[ab]
WH32K72	8.5 (0.5)[b]	1.3 (0.1)[b]	1185 (352)[a]

[a] Fatty acids were removed by hexane extraction of zein isolates. [b] Values in parentheses indicate standard deviation. Within each category, means with no superscript in common are significantly different ($P < 0.05$).

Table 5. Mechanical Properties of Commercial Zein Films[a,b]

plasticizer (15% w/w)[c]	TS (MPa)	ETB (%)	modulus (MPa)
control[d]	10.9 (1.0)[b]	2.4 (0.8)[a]	551 (66)[b]
palmitic acid	9.1 (1.4)[b]	2.4 (0.5)[a]	664 (28)[b]
linoleic acid	9.4 (2.5)[b]	3.3 (0.5)[a]	420 (167)[b]
oleic acid	20.4 (5.3)[a]	2.6 (0.6)[a]	1207 (234)[a]
PEG 400	18.3 (2.5)[a]	3.7 (0.8)[a]	801 (119)[b]

[a] Freeman F-4000. [b] Values in parentheses indicate standard deviation. Within each category, means with no superscript in common are significantly different ($P < 0.05$) [c] Added as percent of the total film-forming solution. [d] No plasticizer.

of the films prepared from zein isolates with nonpolar lipids removed by hexane extraction were significantly lower than thsoe of films containing the endogenous lipids (compare **Tables 3** and **4**). The presence of endogenous FFA for zein isolate films increased ETB values by 40% for films from WH32H39 and by 200% for films from DMC. In addition, zein isolate films prepared from DMC with nonpolar lipids removed were stronger and stiffer than in the presence of lipids, but the results were not as clear-cut for the other zein isolates. Films prepared from commercial zein were also clear and exhibited mechanical properties similar to those of films prepared from DMC with FFA removed (compare **Tables 4** and **5**). Addition of 15% palmitic acid resulted in cloudy, nonhomogeneous films, with mechanical properties similar to the control. Films plasticized with linoleic acid, the most abundant FFA that accumulates in ground corn, exhibited TS and modulus values similar to films containing palmitic acid but were clear. Addition of 15% oleic acid or PEG resulted in stronger films than films plasticized with palmitic or linoleic acid or the control. The TS values of zein isolate films from freshly ground and preground corn stored for 112 days were similar (**Table 6**); however, films prepared from zein isolated from preground corn exhibited elongation >3 times and modulus less than half the values for those films prepared from freshly ground corn. These changes can probably be attributed to the hydrolysis of TAG to FFA and DAG in the stored preground corn (**Figure 1**).

These results indicate that zein isolates are an inexpensive and viable replacement for commercial zein. Dickey et al. (*12*) estimated the cost of the ethanol extraction of the isolate from ground dent corn to be significantly cheaper than that from commercial purified zein. The blend of lipids in the isolate permits casting of clear zein films, and the presence of endogenous FFA obviates the need for additional plasticizers. Storage studies indicated that zein isolates with different film-forming properties could be prepared from preground corn stored under controlled conditions.

Table 6. Mechanical Properties of Zein Isolate Films from Freshly Ground and Preground Corn[a,b]

corn	TS (MPa)	ETB (%)	modulus (MPa)
freshly ground	8.9 (3.8)[a]	1.4 (0.2)[b]	868 (275)[a]
preground	9.0 (0.7)[a]	4.6 (0.1)[a]	408 (49)[b]

[a] Corn YH34K77 was stored for 112 days at 26.8 ± 0.2 °C and 17 ± 1% RH. [b] Values in parentheses indicate standard deviation. Within each category, means with no superscript in common are significantly different ($P < 0.05$).

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