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Recovery and Characterization of α-Zein from Corn Fermentation Coproducts

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ABSTRACT: Zeins were isolated from corn ethanol coproduct distiller's dried grains (DDG) and fractionated into α- and $\beta\gamma$-rich fractions. The effects of the ethanol production process, such as fermentation type, protease addition, and DDG drying temperature on zein recovery, were evaluated. Yield, purity, and molecular properties of recovered zein fractions were determined and compared with zein isolated from corn gluten meal (CGM). Around 29−34% of the total zein was recovered from DDG, whereas 83% of total zein was recovered from CGM. Process variations of cooked and raw starch hydrolysis and fermentation did not affect the recovery, purity, and molecular profile of the isolated zeins; however, zein isolated from DDG of raw starch fermentation showed superior solubility and film forming characteristics to those from conventional 2-stage cooked fermentation DDG. Protease addition during fermentation also did not affect the zein yield or molecular profile. The high drying temperature of DDG decreased the purity of isolated zein. SDS-PAGE indicated that all the isolated α-zein fractions contained α-zein of high purity (92%) and trace amounts of β and γ-zeins cross-contamination. Circular dichroism (CD) spectra confirmed notable changes in the secondary structure of α-zeins of DDG produced from cooked and raw starch fermentation; however, all the α-zeins isolated from DDG and CGM showed a remarkably high order of α-helix structure. Compared to the α-zein of CGM, the α-zein of DDG showed lower recovery and purity but retained its solubility, structure, and film forming characteristics, indicating the potential of producing functional zein from a low-value coproduct for uses as industrial biobased product.

KEYWORDS: corn, zein, prolamins, proteins, biofuels, distiller's grains, DDG, corn biorefinery

INTRODUCTION

Zeins, prolamins of corn endosperm, are composed of 60% of the endosperm protein and 52% of the total kernel proteins.[1] The zein proteins are soluble in aqueous alcohol and classified into four classes (α, β, γ, and δ) based on their solubility, molecular weight, and structure.[2,3] The major zein protein, α-zein, makes up ∼80% of total zeins and consists of two proteins, Z19 and Z22, with apparent molecular weights of 22 and 24 kDa, respectively. β-Zein is ∼10% of total zein and consists of a single 17 kDa subunit. γ-Zein consists of two subunits γ_1 and γ_2 with molecular weights of 27k and 18k, respectively. δ-Zein is a single polypeptide of 10 kDa molecular weight existing in minor quantities.[4,5] In native zeins, β and γ zeins are cross-linked by disulfide bonds, and these two proteins are less hydrophobic than α-zein. These structural variations influence their solubility: α-zein is soluble in 60−95% aqueous ethanol, but β and γ zeins are soluble at 60% aqueous ethanol but not in 90% aqueous ethanol.[3,6] On the basis of differences in solubility, the zeins are isolated and can be utilized in various applications such as paper, paint, fiber, textile, packaging, and biodegradable composite.[7,8] Commercial zein is typically isolated from corn gluten meal (CGM), a protein-rich coproduct of corn wet mills. For research purposes, zein proteins have also been extracted as a coproduct from the dry-grind ethanol process as a front-end recovery prior to converting starch to ethanol.[9−11]

In 2010, 4.2 billion bushels of corn was utilized as a feedstock to produce fuel ethanol in over 200 ethanol plants across the United States.[12] Of the total production of grain-based fuel ethanol, 85% is produced in dry-grind plants and the rest is through wet-mill refineries. In the dry-grind process, starch in the corn kernels is converted to ethanol and other nonfermentable components such as protein, oil, and fiber are concentrated in the ethanol coproduct distiller's dried grains (DDG) or distiller's dried grains with solubles (DDGS). Large quantities of the DDGS are produced each year as a low-value coproduct: around 30.9 billion tons of DDGS was produced in 2009 alone in the United States.[12] To use all of the grain components effectively, several research efforts have focused on recovering oil, protein, and fiber/cellulose at either the front-end or the tail-end of the ethanol production process.[13−17]

Recovering proteins at the front end, before fermentation, produces high-quality zein; however, it produces zein with very low yields (2−5% w/w) and requires a large quantity of solvents because the dry-ground corn contains only 8−11% protein.[1] On the other hand, recovering zein from DDG at the tail end of ethanol production is attractive because the mass is greatly reduced and constituents concentrated after having removed starch. Typical DDG contains 31% crude protein.[18] However, there are several issues that need to be considered when designing effective tail-end recovery strategies. First, the high-temperature conditions used in starch liquefaction and gelatinization could be detrimental to zein recovery and its end use. Some modern ethanol plants use cold/raw fermentation instead of hot gelatinization, but they typically use protease to cleave the protein matrix surrounding starch granules. The effect of protease treatment on zein in DDGS is unknown. Second, the

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fraction remaining after distilling ethanol from the fermented beer is separated into wet cake and thin stillage (soluble syrup). The soluble syrup contains soluble nonzein proteins, free oil, minerals, and residual saccharides and is usually added back to wet cake after concentrating it by evaporation. Adding soluble syrup to wet cake improves its feed quality, but their effect on zein recovery from solids is uncertain. Third, distiller's grain is dried at high temperatures ranging from 120 to 600 °C depending on the ethanol plant; the effects of the higher drying temperature on zein and its recovery are unknown.

The objective of this study was to determine how the following ethanol process variations influence tail-end zein recovery and its quality: (i) fermentation type (cooked/raw starch fermentation), (ii) soluble syrup addition to distiller's dried grains (DDG/DDGS), (iii) protease addition during fermentation, and (iv) DDG drying temperatures. The recovery, purity, and molecular characteristics of the zein, including film forming property, were determined and compared to CGM zein.

MATERIALS AND METHODS

Corn Samples. Yellow dent corn was obtained from the Heart of Iowa Cooperative (Nevada, IA). The enzymes α-amylase SPEZYME (13 642 α-amylase units/g) and G-ZYME (401 gluco-amylase units/g) were obtained from Genencor International (Cedar Rapids, IA). Ethanol Red, dry yeast *Saccharomyces cerevisiae*, was obtained from Fermentis, Lesaffre Yeast Corp. (Headland, AL). Lactrol (462 g virginiamycin/lb), an antibiotic extract, was obtained from PhibroChem (Ridgefield Park, NJ). Stargen, a mixture of enzymes containing *Aspergillus kawachi* α-amylase and glucoamylase that synergistically hydrolyze raw/granular starch to glucose), and FermGen (protease) were provided by Genencor International (Palo Alto, CA).

Feedstock Preparation. Four types of feedstocks were prepared for zein extraction: (i) corn gluten meal by wet-milling (CGM), (ii) DDG of conventional two-step cooking, saccharification, and fermentation (DDG cooked), (iii) DDG of raw starch hydrolysis−fermentation without protease addition (DDG raw − protease), and (iv) DDG of raw starch hydrolysis−fermentation with protease addition (DDG raw + protease). Corn gluten meal was produced by using a 1-kg laboratory wet-milling procedure described by Vignaux et al.[19] All DDG were produced by laboratory-scale fermentation, which closely simulated the industrial fermentation process as described in Paraman et al.[20] Same variety of corn was used to prepare all the CGM and DDG to avoid the effect of varietal differences of corn on zein recovery and properties. Typically, CGM and DDG were dried at 50 °C in a forced air oven at 50 °C for 12 h. In experiments that studied the effect of DDG drying temperature on zein recovery, the DDGs were dried at three different temperatures (27, 50, and 100 °C) to a final moisture content of 4−6%.

Zein Extraction. Zein proteins were extracted using an optimized zein extraction procedure described by Anderson and Lamsal[16] and outlined in Figure 1. Briefly, 25 g of DDG or CGM was extracted with 150 g of 70% (v/v) aqueous ethanol containing 0.5% sodium hydroxide and 1% sodium bisulfite. The extraction was carried out in tightly closed 250-mL centrifuge bottles at 60 °C for 2 h in a water bath with continuous mixing by a magnetic stirrer placed underneath the water bath. The solution was centrifuged for 10 min at 4000 × *g* (Beckman, Palo Alto, CA) at room temperature, and the supernatant was decanted into a 1 L centrifuge bottle. While mixing gently, four volumes (∼500 mL) of 100% ethanol was added to the supernatant to increase the ethanol concentration from 70% to 90% (v/v). The solution was stirred for 30 min at room temperature and centrifuged at 2000 × *g* 10 min to recover the pellet. The pellet (β and γ zein) was dried in a vacuum oven. The supernatant was kept at −18 °C overnight to allow

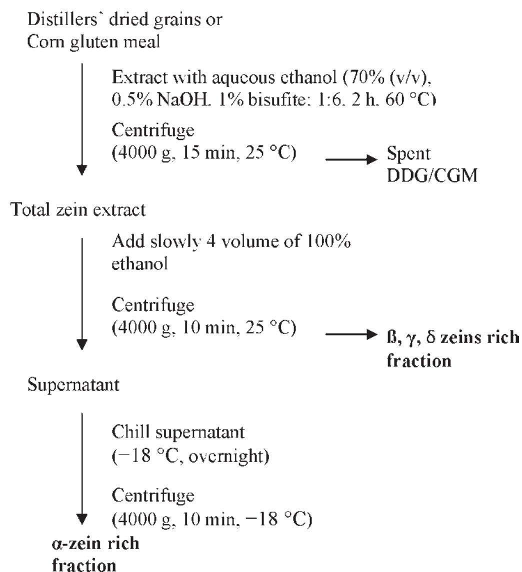

Figure 1. Procedure for recovering α- and βγ-rich zein proteins from corn gluten meal and distiller's dried grains.[16]

α-zein to precipitate. The α-zein was recovered by centrifuging at 2000 × *g* at −18 °C for 10 min, the supernatant was discarded, and the pellet (α-zein) was again dissolved in 50 g of 90% aqueous ethanol (v/v) and dried in a vacuum oven at 50 °C and 0.6 bar for 48 h. The purpose of the redissolving step was to completely transfer all the precipitated α-zein from the centrifuge bottle to the drying container.

Zein yield was expressed as a percent of initial mass of starting material used to isolate zein; α-zein recovery was expressed as a percent of α-zein recovered from the amount of total zein in the starting material

Chemical Analysis. The moisture contents of all samples were determined using the 130 °C convection oven method of AACC 44-19.[21] Crude protein contents were determined using the Dumas nitrogen combustion method with an Elementar Vario MAX CN analyzer (Elementar Analysesysteme GmbH, Hanau, Germany); the conversion factor for protein was 6.25 × N. Total zein contents in whole corn, CGM, and DDG were determined by the method described by Wu et al.[22] using 55% (v/v) isopropyl alcohol, 5% (v/v) dithiothreitol (DTT), and 0.5% (w/v) sodium acetate as the solvent system after sequentially removing water- and salt-soluble proteins.

SDS-PAGE. The molecular weight profile of corn samples, coproducts, and the extracted zein samples were determined by SDS-PAGE with a 4% stacking gel and 12% separating gel in an SDS−Tris−Glycine buffer system. Corn, CGM, and β and γ-zein samples were prepared on a protein basis (2−4 μg protein/μL) in reducing sample buffer, containing 125 mM Tris-HCl at pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol, and 0.05% bromophenol blue. α-Zein samples were dissolved in 70% (v/v) aqueous ethanol and diluted to 2−4 μg protein/μL with the above sample buffer. All protein solutions were centrifuged at 5000 × *g* for 2 min to remove insolubles, and 16 μL of the soluble protein was loaded onto the gel. Electrophoresis was performed at a constant voltage of 200 V for 40 min. The gel was stained by 0.1% Coomassie brilliant blue solution. Bio-Rad molecular weight standards ranging from 10 to 200 k were used.

Circular Dichroism Spectroscopy. Secondary structure contents of the zeins isolated from CGM and DDG were determined by CD spectroscopy. The α-zein protein solutions were made in 70% ethanol to a final concentration of 5 μg/mL and filtered through a 0.22 μm membrane (Millipore Corp., Milford, MA). The CD spectra of α-zein samples were scanned in the far-UV ranging from 260 to 190 nm on a Jasco J-710 spectropolarimeter using a 0.1 cm path length quartz cell.

Table 1. Composition of CGM and DDG of Cooked and Raw Fermentation[a]

source	crude protein content (%)	total available zein (%)[b]	zein of total protein (%)[c]	crude free fat (%)
CGM	49.6 ± 3.0	33.7 ± 4.2	68.6 ± 12.9	2.6 ± 0.9
DDG cooked fermentation	34.7 ± 2.6	19.0 ± 3.4	54.6 ± 7.7	5.6 ± 1.0
DDG raw fermentation − protease	32.5 ± 2.5	17.4 ± 2.3	53.6 ± 6.8	6.9 ± 1.5
DDG raw fermentation + protease	29.1 ± 1.4	15.9 ± 2.7	54.7 ± 11.5	6.3 ± 1.2
commercial DDGS-defatted	32.0 ± 0.5	17.0 ± 3.7	53.1 ± 10.9	2.9 ± 0.7

[a] CGM, corn gluten meal; DDG, distiller's dry grains. Values are means ± standard deviations of three replicates on dry weight basis. [b] On the basis of the weight of starting material. [c] On the basis of crude protein content.

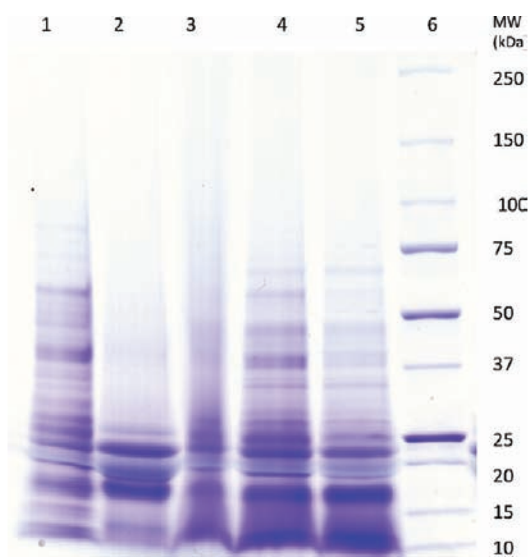

Figure 2. SDS-PAGE protein profile of corn and its coproducts: ground whole kernels (lane 1); wet-milled CGM (lane 2); cooked-DDG (lane 3); raw DDG-protease (lane 4); raw DDG + protease (lane 5); molecular weight marker (lane 6).

The spectra were corrected for baseline and dissolving solvent, 70% ethanol. The molar ellipticity $[\theta]$ was calculated using a value of 109.8 g/mol for the molecular weight of the mean residue of zein.[23]

Film Properties. Zein films were made by the method described by Parris et al.[24] with some changes. One gram of α-zein was dissolved in 25 mL of 90% (v/v) aqueous ethanol by mixing it for 4 h at room temperate in a tightly closed container. Then the solution was heated at 50 °C for 10 min with stirring and cast in polystyrene Petri dishes. The films were dried in the vacuum oven at 50 °C at 0.6 bar for 2 h. For tensile tests, the films were cut into 5-mm wide dumbbells. The exact width and thicknesses of the film specimens were measured using a digital micrometer. Tensile properties the films were estimated by an Instron model 1122 tensile tester using a gauge length of 25 mm and an extension rate of 1 mm/min.[25]

Statistical Analysis. Experiments were replicated three times at the starting point of CGM and DDG preparation. Data were analyzed using analysis of variance with JMP v. 8.0.1 statistical software (SAS Institute, Inc., 2010). The least significant differences were determined using the Tukey−Kramer HSD test at the 5% significance level.

RESULTS AND DISCUSSION

Zein Composition and Protein Profile. Table 1 shows the zein and total protein contents of CGM and DDG preparations. Of the total protein, CGM and DDG contained 68% and ~53% zeins, respectively. In CGM, zein proteins are highly concentrated (Figure 2, lane 2) because germ proteins and other soluble proteins were removed in wet-milling process used to produce CGM. Zein in DDG is less concentrated than in CGM but more concentrated than in whole corn. Among the DDGs, the protein profiles were very similar regardless of process variations used in fuel ethanol production such as cooked/raw starch fermentation or protease addition (Figure 2).

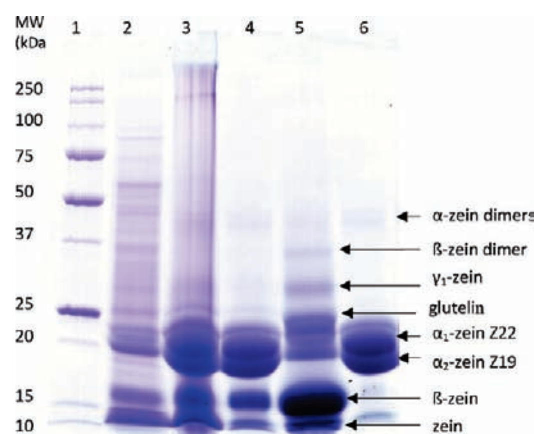

Figure 3. Protein profile of zein fractions isolated from corn gluten meal (CGM): molecular weight marker (lane 1); ground whole kernels (lane 2); wet-milled CGM (lane 3); total zein (lane 4); $\beta\gamma$-rich zein (lane 5); α-zein (lane 6).

Importantly, zein proteins, particularly α-zein, were not hydrolyzed by the protease FermGen used in fermentation (Figure 2, lane 5). The resistance of zeins to protease might be due to the fact that α-zeins are highly hydrophobic and packed in the interior of the protein bodies.[1] However, protease hydrolyzed the non-zein proteins and thus produced notably lower intensity bands for these proteins (Figure 2, lane 5) than those in DDG produced without protease addition (Figure 2, lane 4).

Table 1 also confirmed that all DDG contain a similar amount of total available zein: DDG produced with protease treatment (15.9%) and other DDGs produced without protease treatment (17.4%). However, as indicated in Table 1, the total protein content was lower in DDG + protease (29%) than DDG − protease (33%), probably due to the loss of hydrolyzed proteins removed with the soluble (thin-stillage) fraction.

Zein Recovery. The solvent system extracted all fractions of zein protein: α, β, γ, and δ zeins. Use of a reducing agent, sodium bisulfite, facilitated extraction and fractionation of the zeins based on their solubility difference.[6] β- and γ-zeins were first precipitated from the extract by increasing the ethanol concentration from 70% to 90%. Electrophoresis of the β- and γ-zein fraction indicated the glutelin proteins were also coprecipitated with β- and γ-zeins (Figure 3, lane 5). When the α-zein was recovered by cold precipitation, the recovered α-zein was high in purity

(92%) and contained highly concentrated α_1 and α_2 zeins having M_r of ~22 and 24 kDa, respectively (Figure 3, lane 6) and the α-zein fraction contained only a trace quantity of β- and γ-zeins at 15 and 10 kDa, respectively. Separating the other zeins from α-zein improves the film forming capabilities of the isolated polymer, and other zeins promote gel formation at high temperatures.[8]

It is important to note that the zeins isolated from different types of DDGs (cooked DDG and raw DDG with/without protease) showed remarkably similar molecular profiles (Figure 4), a protein profile similar to CGM zein. The protein profile of α-zein indicated the majority of the zeins were not hydrolyzed or degraded during the ethanol production process, a vigorous process that uses crude enzymes and high temperatures.

Although DDG contained several proteins and other soluble substances, the isolated α-zein showed a remarkably high protein content (88%) with high protein purity (92%). The improved purity is attributed to the two-stage precipitation involved in zein recovery (Figure 1). First, β- and γ-zein were precipitated by increasing the ethanol concentration to 90%. Considerable amounts of other insoluble impurities were coprecipitated and removed with β- and γ-zeins, as it showed a low protein (~60%) presence, confirming the coprecipitation of impurities. Second,

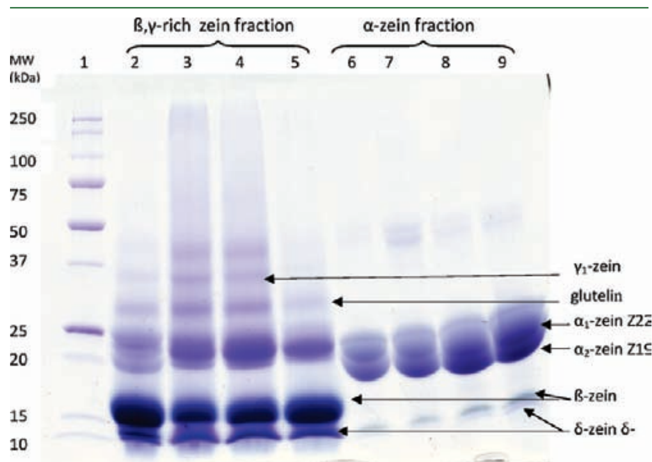

Figure 4. Protein profile α- and β γ-rich zeins isolated from CGM and DDG: molecular weight marker (lane 1); β γ-zein of CGM (lane 2); β γ-zein of cooked DDG (lane 3); β γ-zein of raw DDG-protease (lane 4); β γ-zein of raw DDG + protease (lane 5); α-zein of CGM (lane 6); α-zein of cooked DDG (lane 7); α-zein of raw DDG-protease (lane 8); α-zein of raw DDG + protease (lane 9).

when α-zein was precipitated by chilling the solution to −18 °C overnight, any soluble impurities possibly remained in the solution, making it possible to recover α-zein of high purity containing ~88% protein.

Effect of Stillage Solubles on Zein Recovery. To determine how the presence of soluble constituents in syrup produced during fermentation affects zein recovery, DDG and DDG with soluble (DDGS) were produced and used as starting material for zein recovery. Yield, recovery, and protein content of α-zein isolated from DDG and DDGS are summarized in Table 2. Both DDG and DDGS produced similar yields of α-zein from both cooked and raw fermentation processes. Zein recoveries were also similar for both DDG and DDGS. However, the recovered α-zein fraction of DDG contained higher protein contents (86−88%) than that from DDGS (71−77%). The lower protein content of the zein isolated from DDGS indicated that a considerable amount of soluble substances was coextracted with α-zein.

Separating the soluble fraction (thin stillage), before drying the distiller's grains, improved the protein content of the α-zein isolated from the DDG. In the dry-grind ethanol process, whole corn is ground and used for fermentation without separating germ; thus, the free oils and soluble germ proteins end up with the soluble fraction. The soluble substances possibly cross-linked to α-zein particularly with high-temperature drying and produced zein with lower purity. We observed that the isolated α-zeins of DDG had a smoother and less brownish appearance than that of DDGS (pictures not shown). Removing solubles before drying also adds a processing advantage of eliminating the defatting step in tail-end zein recovery because preliminary observations indicated that defatting DDGS improved the purity of the isolated zein from 71% to 80% but defatting DDG only improved the purity from 87% to 89% (data not shown).

Effect of Fermentation Type on Zein Recovery. Table 3 indicates the yield and recovery of α-zein isolated from DDGs produced from various ethanol production processes: (i) cooked/raw fermentation, (ii) with/without protease addition, and (iii) low/high temperature of DDG drying. Overall, yields of zein isolated from DDG (5.7−7.2%) were much lower than that from CGM (30.8%); the lower recovery of zein might be due to not only lower extractability of zein from DDG but also lower initial zein contents in DDG (15−18%) compared to CGM (33.7%) (Table 1). Thus, zein recoveries were calculated based on the initial zein content to determine how zein content affects the extractability.

Unexpectedly, the zein recovery did not vary with various types of fermentation used in today's ethanol industries: cooked

Table 2. Yield, Protein Content, and Recovery of α-Zein Isolated from DDG and DDGS[a]

source/drying temp.	α-zein yield (%)[b]	α-zein protein content (%)	α-zein recovery (%) (based on total zein in substrate)[c]
		cooked fermentation	
DDGS	7.0 ab	71.5 c	33.9 a
DDG	7.3 a	86.4 a	33.7 a
		raw fermentation + protease	
DDGS	6.4 bc	77.1 b	32.7 ab
DDG	6.0 c	87.2 a	31.5 ab
commercial DDGS defatted	5.9 c	83.5 a	29.8 b

[a] DDG and DDGS were produced in house and dried in a forced air oven at 50°C. Zein was extracted with a solvent system containing 70% ethanol, 1% sodium bisulfite, and 0.5% NaOH. Means in the same column followed by the same letter are not significantly different ($p < 0.05$). [b] Calculated based on the weight of starting material. [c] Calculated based on total zein content of the starting material.

Table 3. Effect of Fermentation Process and DDG Drying Temperature on Yield, Protein Content, and Recovery of Isolated α-Zein[a]

source/drying temp.	α-zein yield (%)[b]	α-zein protein content (%)	α-zein recovery (%)[c]
corn gluten meal	30.8 a	91.1 a	83.3 a
DDG cooked fermentation			
28 °C air drying	7.2 b	85.7 b	33.2 b
50 °C oven drying	7.3 b	86.4 b	33.2 b
100 °C oven drying	6.9 b	73.6 c	29.2 bc
DDG raw fermentation − protease			
28 °C air drying	6.1 bc	88.6 b	31.3 b
50 °C oven drying	5.9 bc	88.0 b	29.8 b
100 °C oven drying	5.8 bc	77.1 c	26.5 c
DDG raw fermentation + protease			
28 °C air drying	5.9 bc	87.9 b	32.4 b
50 °C oven drying	6.0 bc	88.2 b	33.2 b
100 °C oven drying	5.7 c	78.3 c	25.8 c
commercial DDGS defatted[d]	5.9 bc	83.5 bc	29.0 bc

[a] Zein recovery was calculated based on the total zein contents of 68% in CGM protein and 53% in DDG protein. Means in the same column followed by the same letter are not significantly different ($p < 0.05$). [b] Calculated based on the weight of starting material. [c] Calculated based on total zein content of the starting material. [d] Commercial DDGS was obtained from Lincolnway Energy, Ames, IA.

and raw fermentation process or even with protease addition during fermentation (Table 3). We anticipated the zein extractability to be low for cooked-DDG because zein denatures irreversibly and forms aggregates via hydrophobic and disulfide interactions upon heating.[23] The use of sodium bisulfite at high concentration (1%) might have facilitated extraction of zein from DDG, which cross-linked or aggregated as a result of high temperatures used in various stages of the ethanol production process such as liquefaction, gelatinization, and drying. Partial reduction of disulfide bonds with bisulfite might have improved the extractability of zein from DDG.

Raw DDG produced by cold fermentation yielded almost the same amount of zein as obtained from cooked DDG (Table 3). We expected to recover zein with a higher yield from the raw DDG because this ethanol production process does not use a high-temperature gelatinization process; we hypothesized that zein would be in its native state, without thermally induced cross-links and protein aggregates. Zein recovery was also not affected by adding protease to the fermentation. As described earlier, α-zeins are located inside of protein bodies[1] and highly hydrophobic and thus not hydrolyzed by the protease used in fermentation. This confirms the observations of Lawton and Freeman,[26] who also reported that corn protein bodies remain intact during the dry-grind ethanol process, and the process had not affected zein extraction yields from DDG of raw and cooked processes. The higher zein recovery achieved in this present study might be due to the use of a high concentration of bisulfite (1%), as supported by use of breakdown agent NaOH in the study by Lawton and Freeman,[26] and removal of the soluble syrup prior to drying of DDG.

Effect of DDG Drying Temperature on Zein Recovery. The isolated zeins of CGM contained a higher protein content (91%) than that of DDG (74−88%). The high-temperature DDG drying condition decreased the recovery of zein and lowered the purity of the isolated zein (Table 3). α-Zein isolated from DDG dried at 28 and 50 °C contained a higher protein content (85−89%) than that dried at 100 °C (74−78%), confirming the cross-linking of zein with nonprotein components such as sugars and carbohydrate during high-temperature drying of DDG.

The commercial DDGS was obtained from a dry grind ethanol facility (Lincolnway Energy, Ames, IA). Commercial plants typically dry the DDGS at 120−600 °C, but the actual contact time at that temperature is short, only 2−3 min. In lab experiments, DDGs were dried at three different temperatures (27, 50, and 100 °C) for varying times (2−24 h) to a final moisture content of 4−6%. It was, therefore, not possible to compare the effect of actual commercial and laboratory drying conditions because of the huge differences in drying conditions. The DDG dried at varying temperatures showed a clear difference in end-product color, and the data clearly indicated that the high drying temperature conditions had an adverse effect on zein recovery and its end purity (Table 3). Zein isolated from DDG that dried at 100 °C had a burned appearance, but the zein of low-temperature drying produced zein with a clear colorless appearance (data not shown).

Table 4 indicates that the isolated α-zein from DDG had good resolubility properties, ranging from 83% to 92%, which was lower than that of CGM α-zein (96%). Among the DDG zeins, the α-zein isolated from raw starch fermentation DDG showed better solubility (92%) than that of cooked DDG (83−88%). Parris et al. (2001) indicated that bisulfite treatment considerably improved the solubility without impairing its film forming properties.[6]

Secondary Structure of α-Zein. The changes in the secondary structures of the isolated α-zeins were determined by CD spectroscopy. Figure 5A indicates the far-UV−CD spectra (200−260 nm) of the α-zein proteins solubilized in 70% aqueous ethanol. Two negative maxima observed at 208 and 222 nm indicated the presence of α-helix secondary structure, and the intensity of the peaks reflected the amount of α-helix structure present in these proteins.[27−29] The CD spectra showed clear differences in the secondary structure of α-zeins isolated from various DDGs. In addition to process variation, the degree of zein purity might have also contributed to the structural variations in these samples, because the protein purity must be

Table 4. Solubility and Film Characteristics of α-Zein Isolated from CGM and DDG[a]

treatment	solubility (%)	tensile strength (MPa)	elongation to break (%)	appearance
CGM	96.2 a	26.3 a	2.1 a	smooth/clear/light yellow
DDG cooked fermentation	83.6 c	18.9 bc	0.9 c	rough/cloudy/light brown
DDG raw fermentation − protease	89.5 bc	24.5 ab	1.5 b	smooth/clear/colorless
DDG raw fermentation + protease	92.1 b	20.6 b	1.2b c	smooth/clear/colorless
commercial DDGS-defatted	88.0 bc	16.3 c	1.4 b	rough/cloudy/light brown

[a] Means in the same column followed by the same letter are not significantly different ($p < 0.05$).

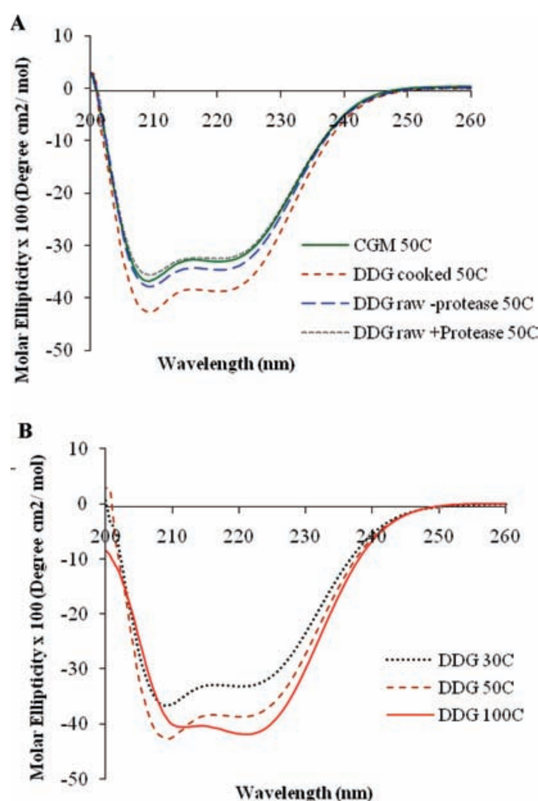

Figure 5. (A) Far-UV−CD spectra for α-zein isolated from CGM and DDG of cooked and raw fermentation. (B) Far-UV−CD spectrum of α-zein isolated from DDG dried at various temperatures.

at least 95% for accurate quantification of the secondary structure.[29] Our zein proteins had only 85−91% purity, and thus, secondary structures quantified from those samples might not be highly accurate. However, CD spectra provided relative information on how secondary structure contents changed as a result of the high temperatures used in fuel ethanol production.

Previous studies indicated that zein irreversibly denatured and formed random coils and turns on heating at 90 °C in 70% ethanol.[23] However, in the present study, the zein isolated from cooked DDG showed high contents of α-helix, almost similar to that observed for CGM and raw DDG. The exact reason for this discrepancy was not clear. On the basis of Figure 5B, it appeared the isolated zein retained a high order of α-helix structure even after the extensive heating used in ethanol production processes. The use of a high concentration of bisulfite in our zein extraction probably cleaved the thermally formed disulfide bonds and facilitated the return of α-zein to its initial secondary structure.

Film Characteristics of α-Zein. The film forming properties of α-zein isolated from DDG were evaluated and compared with those of CGM zein. Tensile strength, elongation-to-break values, and visual appearance are presented in Table 4. CGM zein formed clear, smooth, and flexible films. DDG zeins showed comparatively less smoothness and flexibility than CGM zein. When casting the DDG zein, some insoluble aggregates were observed and were removed by filtration. Among the DDG zeins, a superior quality of zein film was obtained from zein of raw fermentation DDG without protease compare to those from raw fermentation with protease or cooked-fermentation DDG (Table 4). Zein of cooked DDG exhibited a lower tensile strength and lower elongation to break values compared to raw DDG zein. The high-temperature conditions in cooked fermentation might have affected the zein film properties. Despite the variations in film characteristics of CGM and DDG zeins, it is important to note that zein isolated from all DDG retained its film forming properties.

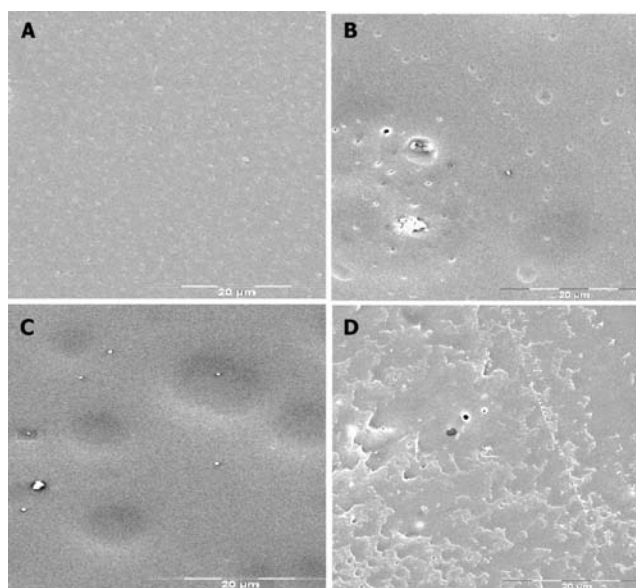

Figure 6. Scanning electron micrographs of film produced from α-zeins CGM and DDG: (A) CGM; (B) cooked DDG; (C) raw DDG − protease; (D) raw DDG + protease.

SEM micrographs (Figure 6) are in agreement with the above observations. CGM film showed a continuous film with a smooth appearance; cooked-DDG zein films contained minute pores on the surface. Raw DDG zein with protease treatment produced a continuous clear film but showed minute projections on its surface (Figure 6D). When casting the films, vacuum oven drying produced films with a superior appearance than drying them in a convection oven or air drying, which formed opaque film probably due to oxidation of sulfhydryls cleaved by bisulfites used in zein extraction.

In this study, we demonstrated that functional α-zein could be isolated from corn ethanol coproduct, DDG. Improved zein recovery (26−33%) and purity (77−89%) were achieved by removing soluble components before DDG drying and using low to moderate drying temperatures. Fermentation type (cooked/raw process) did not affect the zein recovery, but α-zein from raw fermentation DDG produced films with better tensile characteristics than that from cooked fermentation DDG. Overall, the α-zein of DDG showed lower recovery and purity than the zein from CGM; however, the α-zeins of DDG retained their solubility, structure, and film-forming characteristics, suggesting the potential of isolating zein from ethanol coproducts and utilizing them in nonfood, industrial applications.

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