

Development of New Method for Extraction of α -Zein from Corn Gluten Meal Using Different Solvents

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ABSTRACT

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A modified procedure for the extraction of α -zein from corn gluten meal was developed and compared against a commercial extraction method. The modification involved raising the concentration of alcohol in solvent and removing the precipitate by centrifugation. Five organic solvent mixtures were compared using the modified extraction procedure developed along with the reductant sodium bisulfite and NaOH. The modified procedure precipitated most of the non- α -zein protein solids by increasing the concentration of alcohol. The supernatant had α -zein-rich fraction, resulting in higher yield of α -zein than the commercial method when cold precipitated. The commercial extraction procedure had a zein yield of 23% and protein purity of 28% using 88% 2-propanol solvent.

The three best solvents, 70% 2-propanol, 55% 2-propanol, and 70% ethanol, yielded \approx 35% of zein at protein purity of 44% using the modified extraction procedure. Zeins extracted using the novel method were lighter in color than the commercial method. Densitometry scans of SDS-PAGE of α -zein-rich solids showed relatively large quantities of α -zein with apparent molecular weights of 19,000 and 22,000 Da. The α -zein-rich solids also had small amounts of δ -zein (10,000 Da) because it shares similar solubility properties to α -zein. A solvent mixture with 70% 2-propanol, 22.5% glycerol, and 7.5% water extracted significantly less zein (\approx 33%) compared to all other solvents and had α -zein bands that differed in appearance and contained little to no δ -zein.

Zein is \leq 52% protein by weight in the corn kernel. Zein proteins were first described by Gorham (1821), who extracted them from Indian corn. Interest in the protein developed when Osborne (1891) extracted zein from corn gluten meal (CGM), which was a high-protein coproduct of corn wet milling. Later, Osborne and Mendel (1914) classified protein from corn into four different categories based on solubility. These proteins were albumins (soluble in pure water), globulins (soluble in aqueous salt solutions), prolamins (soluble in 70% ethanol), and glutelins (soluble in dilute acid or base). Zein proteins are prolamins and have been further characterized into four different classes based on solubility, electrophoresis, and immunological studies. Esen (1987, 1990) classified them as α , β , γ , and δ -zein. Important past applications of zein were in inks, adhesives, coatings, plastics, and chewing gums (Sturken 1938; Coleman 1939, 1941; Lougovoy 1949; Simonds et al 1949). New potential applications of zein include packaging, carrier material for chemical or drug delivery, and biomedicine such as cellular scaffolds to accelerate cell growth in tissue and bone, while degrading after healing (Dong et al 2004; Wang et al 2007; Tu et al 2009; Jiang et al 2010). It is important to recover higher amounts of functional zein fractions with higher purity to suit potential novel applications.

The first commercial extraction of zein was made in 1939 (Shukla and Cheryan 2001). A refined patent detailed the commercial extraction process for zein using 85% aqueous 2-propanol at 60°C (Swallen 1942). The extract was then treated with hexane to remove pigment; the zein was collected by precipitation in cold water and drying on ring dryers. A current commercial method by Carter and Reck (1970) extracts zein from CGM with 88% (w/w) 2-propanol at 65°C with agitation. The extract is cold-precipitated at -10 to -20°C to precipitate α -zein and dried in a vacuum oven. To produce higher purity zein protein, the cold-precipitated wet solids could be redissolved in the extracting solvent and reprecipitated. The zein extraction method of Carter and Reck (1970) also used 0.25% NaOH to adjust the CGM extraction from pH 6.5

to 7.0, which is near the isoelectric point of many zein phenotypes, causing them to become insoluble while leaving α -zein soluble (Cook et al 1996; Carter and Reck 1970). Another commercial extraction procedure was described by Takahashi and Yanai (1994), who extracted α -zein from CGM using 70% (v/v) aqueous acetone at 40°C. The extract was then concentrated and added to absolute acetone to precipitate the α -zein protein.

These current commercial extraction methods extract primarily α -zein, which is soluble in aqueous alcohol solvents at higher alcohol concentrations such as 88% (w/w) 2-propanol (Esen 1987; Kale et al 2007). A major problem with these methods is low zein yields of 22 and 20.4 g/g of CGM (db) reported by Carter and Reck (1970) and Takahashi and Yanai (1994), respectively. These yields are low considering that $>50\%$ of the α -zein is not extracted (Wu et al 1997b). Zein extraction yields could be improved by using lower aqueous alcohol concentrations such as 55% (w/w) 2-propanol. This increase of yield would be due to co-extraction of all zein fractions resulting in lower α -zein purity (Esen 1986). However, lower alcohol concentrations decrease zein solubility and solution stability (Swallen 1942). Other issues with both types of solvents and extraction systems are 1) the large amounts of solvents required, and 2) the energy intensive processes such as solvent concentration, cold-precipitation, and distillation of solvent for recycling.

The purpose of this research was to modify the Carter and Reck (1970) zein extraction procedure to improve the yield and purity of the zein protein using reductant and different solvents. The solvents in this experiment were selected based on known information about the structure of zein protein bodies and solubilities. Zein protein bodies are $\approx 1 \mu\text{m}$, can survive grinding and mild cooking, and subsequently are intact in corn flour (Batterman-Azcona and Hamaker 1998). The making of CGM consists of grinding and low temperature processing that disperses the endosperm starch and protein matrix to produce intact starch and zein protein bodies (Cox et al 1944; Wu et al 1997a). The zein protein bodies consist of a thin layer of β - and γ -zein interconnected by disulfide bonds. This layer sheaths a large proportion of α -zein at the protein body's core (Mohammad and Esen 1990). One of the commercial extraction methods uses 88% (w/w) aqueous 2-propanol, 0.25% NaOH, and no reducing agent (Carter and Reck 1970). The 88% (w/w) aqueous 2-propanol dissolves α -zein across the layer without dissolving β - and γ -zeins (Carter and Reck 1970). For this research, solvents that extract total zein, such as 55% (w/w) aqueous 2-propanol and 70% (v/v) aqueous ethanol, were chosen.

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These two solvents, with the aid of reducing agent, destabilize β - and γ -zeins layer sheathing the protein body and are more efficient in extracting α -zein (Landry et al 1983; Shukla et al 2000). The solvent 70% (w/w) aqueous 2-propanol was chosen to determine whether dissolution of β - and γ -zeins at modest levels could disrupt the protein bodies and in turn extract more α -zein. The mixture of 70% (w/w/w) aqueous 2-propanol, 22.5% glycerol, and 7.5% water was a new ternary zein solvent. This solvent was modeled to evaluate the potential of inclusion of a co-product of biodiesel processing, namely crude glycerol, at 30% (w/w) glycerol/water level. The objectives of this study were 1) to develop an extraction procedure for CGM to extract all zeins and compare zein yields to the commercial method, and 2) to evaluate the efficacy of reducing agent sodium bisulfite in increasing zein yield.

MATERIALS AND METHODS

Corn Gluten Meal

The CGM used in the zein extraction was obtained from Cargill (Eddyville, IA). The particle size distribution of CGM was determined by using a laser diffraction size analyzer (MasterSizer model 2000, Malvern Instruments, Malvern, UK). The relative proportion of different types of protein in CGM was determined following the method of Wu et al (1997b). Briefly, 10 g (db) of CGM in duplicate was first extracted with 250 mL of 0.5M sodium chloride solution in a 400-mL sealed centrifuge bottle for 20 min at room temperature with stirring to recover Saline-soluble proteins. It was then centrifuged at $8,000 \times g$ for 15 min and the supernatant was collected. Zein proteins were then extracted from the solids pellet with 250 mL of 55% (v/v) aqueous 2-propanol and 5% (v/v) 2-mercaptoethanol with 0.5% (w/v) sodium acetate solvent (PMA). The solution was stirred for 2 hr at room temperature and then centrifuged at $8,000 \times g$ for 15 min. The remaining (spent) solids were washed twice with 50 mL of PMA solvent and these washes combined with the first PMA extract. Before protein analysis, the three fractions of saline-soluble proteins, total zein fractions, and spent CGM were measured for total solids based on

total zein extraction data from the work of Wu et al (1997b). The protein content of these three fractions was determined by using the nitrogen analyzer based on Dumas's combustion method.

Extraction of Zein and Comparison of Solvent Systems

Two methods of extraction were employed as outlined in Fig. 1: Carter and Reck's (1970) Method A using 88% (w/w) aqueous 2-propanol (88-IPA), and a laboratory-modified Method B. Method B added a precipitation step to Method A. Solvents were compared for extraction efficiency using Method B: 70% (w/w) aqueous 2-propanol (70-IPA), 55% (w/w) aqueous 2-propanol (55-IPA), 70% (w/w/w) aqueous 2-propanol, 22.5% glycerol, and 7.5% water (70-GLY-IPA), 70% (v/v) aqueous ethanol (70-EtOH), and 70% (v/v) aqueous ethanol with two cold precipitations (70-TCP-EtOH). Both Methods A and B extracted zein from 23.33 g of CGM (db) using 140 g of solvent in a solute-to-solvent ratio of 1:6 at 60°C for 1 hr. Sodium hydroxide, 0.25%, and reductant sodium bisulfite, 0.5%, were added to 140 g of solvent for reductant treatment during some extractions. The zein extraction method of Carter and Reck (1970) also used 0.25% NaOH to adjust the CGM extract to pH 6.5–7.0, which is near the isoelectric point of many zein phenotypes, keeping them insoluble while leaving α -zein soluble (Carter and Reck 1970; Cook et al 1996). The control extraction had no NaOH and sodium bisulfite treatment.

For Method A, after the crude zein extraction, the slurry was centrifuged at room temperature for 15 min at $8,000 \times g$. The extracted crude zein in supernatant was decanted from spent CGM solids and a sample of 7.5 g of crude zein extract was taken to measure solids content. The remaining extract was held at -20°C overnight to precipitate zein protein into a taffy-like layer. This material was then centrifuged at -20°C and $8,000 \times g$ to recover the solid pellet. The pellet was dissolved in 100 g of 88% (w/w) aqueous 2-propanol and dried in a vacuum oven at 50°C at 0.6 bar pressure. The dry α -zein-rich solid was weighed, ground, and stored at 4°C until use. A portion of ground α -zein-rich solids was further dried in a vacuum oven at the same temperature and pressure for four days for moisture determination.

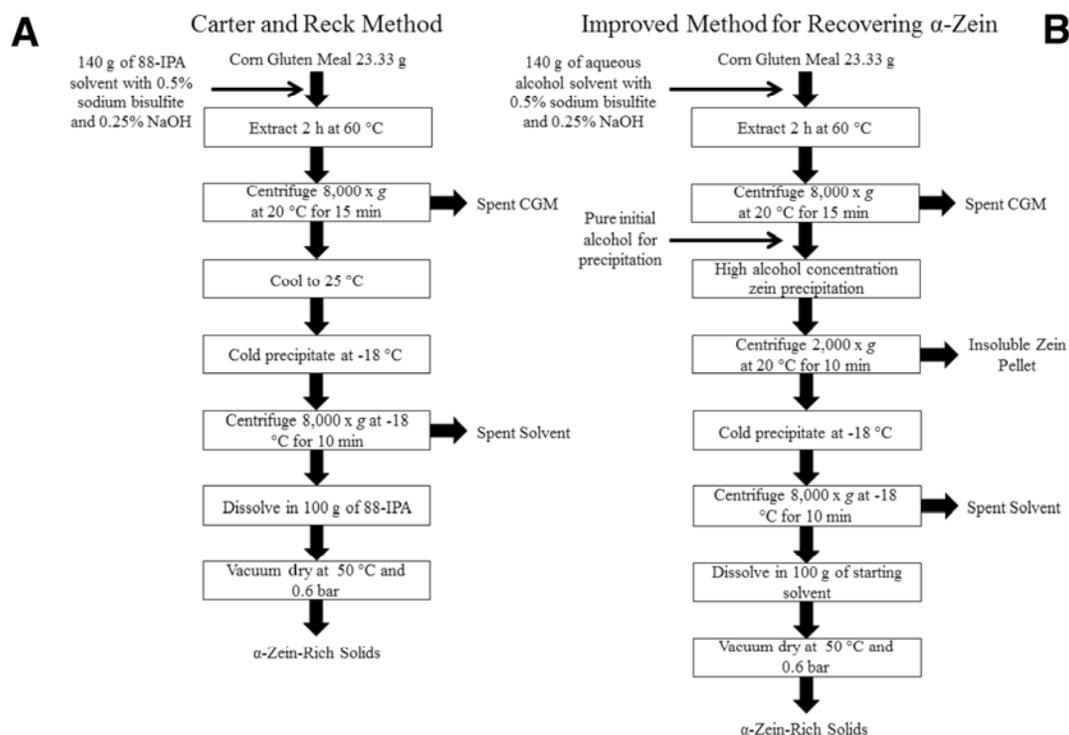


Fig. 1. Method A is Carter and Reck (1970) extraction method (A), Method B is a modification of Method A evaluated in this study with different solvents (B). Method B adds a precipitation and centrifuge step after crude zein extraction.

Method B followed the same crude zein extraction step as in Method A, but used solvents 70-IPA, 55-IPA, 70-GLY-IPA, 70-EtOH, or 70-TCP-EtOH. After extraction and centrifugation, the 2-propanol was added to the supernatant of 70-IPA, 55-IPA, and 70-GLY-IPA solvents until each had a final aqueous concentration of 88% 2-propanol. For the supernatant obtained with 70-EtOH, and 70-TCP-EtOH, alcohol concentrations were increased to 95% (v/v) aqueous ethanol. The amount of alcohol used to increase the solvent concentrations to precipitate β - and γ -zeins was based on the mass of estimated crude zein in the extract from the total zein extractions of Wu et al (1997b). The alcohol was added slowly and continuously. This step precipitated β - and γ -zeins leaving α -zein in solution (Esen 1986; Parris and Dickey 2001). The resulting turbid solution was stirred for 30 min and centrifuged at $2,000 \times g$ for 10 mins at room temperature. The protein pellet of mostly β - and γ -zeins was retained for further analysis. The clear supernatant was held at -20°C overnight to allow protein precipitation. The precipitate was centrifuged at $8,000 \times g$ at -20°C for 15 min, the supernatant was discarded. The α -zein-rich solid was dissolved in 100 g of either 88% (w/w) aqueous 2-propanol or 95% (v/v) aqueous ethanol, based on which alcohol was used for the primary extraction. The dissolved α -zein was placed in a 600-mL beaker and dried in the vacuum oven at 50°C at 0.6 bar (Wu et al 1997b). When dried, α -zein-rich solids recovered were weighed, ground, and stored at 4°C until use. A portion of ground sample was further dried in the vacuum oven at the same temperature and pressure for four days for moisture determination.

Extraction Parameters and Calculations

The extraction terminologies differed for various measured extraction parameters. The term crude zein indicated the recovered protein during the initial extraction of CGM. Crude zein protein, % = [(mass protein extracted)/(total protein in CGM mass)] \times 100. The remaining solids after crude zein extraction were spent CGM. Spent CGM protein, % = [(mass of unextracted protein)/(total protein in CGM mass)] \times 100. In Method B, the insoluble zein pellet was precipitated from the alcohol extract of crude zein, leaving the α -zein-rich proteins in solution. Insoluble zein pellet, % = [(mass protein precipitate in pellet)/(total protein in CGM mass)] \times 100.

Zein yield was the mass of zein extracted relative to the mass of starting CGM. Zein yield, % = [(mass α -zein-rich solids)/(total mass of CGM, db)] \times 100. The protein purity was the protein content of the recovered α -zein-rich solid. The protein recovery is a more accurate final yield parameter for zein, taking into account the percent protein of the α -zein-rich solids relative to the mass of protein in the initial substrate (Wu et al 1997b). Protein recovery, % = (protein purity, %) \times (mass α -zein-rich solids)/(total mass of protein in starting CGM). The α -zein extraction efficiency determined the amount of α -zein recovered at the end of the extraction. α -Zein extraction efficiency, % = (protein purity, %) \times (mass of α -zein-rich solids)/(total mass of α -zein protein in starting CGM). The fraction of α -zein relative to the total zein protein in CGM was determined by using densitometry as described below for SDS-PAGE and densitometry.

SDS-PAGE and Densitometry

The α -zein-rich solids and the total zein samples (Wu et al 1997b) were separately analyzed by SDS-PAGE and densitometry. SDS-PAGE gels were prepared according to methods of Laemmli (1970). Because of the nonpolar characteristics of zein, it was difficult to dissolve α -zein-rich solids in polar sample buffers. To disperse and homogenize the zein protein, the solid was first dissolved in 70% (v/v) aqueous ethanol and then added to a pH 6.8 sample buffer consisting of 3.55 mL of deionized water, 1.25 mL of 0.5M Tris HCl (6.055% aqueous solution of tris(hydroxymethyl)aminomethane and 0.4% SDS), 2.5 mL of glycerol, 2.0 mL of a 10% (w/v) SDS solution, 0.2 mL of a 0.5% (w/v) bromophenol

blue solution, and 0.5 mL of 2-mercaptoethanol. The dissolved zein-to-sample buffer ratio was <0.5 to keep samples from migrating out of the well due to buoyancy. After the dissolved zein was added to the sample buffer, the mixture was heated at 100°C for 5 min and kept at 4°C until applied to the gel. Stacking gels and the resolution gel were prepared at 4 and 13% acrylamide, respectively. For the total zein sample, the same stacking gel concentration was used, but the resolution gel was increased to 15% acrylamide to better resolve the proteins. The α -zein-rich solids sample (40 μL) was loaded at a concentration of 3 $\mu\text{g}/\mu\text{L}$ to the gels. The protein standard was a low molecular weight marker (M-3913, Sigma, St. Louis, MO) consisting of proteins with MW from 6,500 to 66,000 Da. SDS gel images were scanned using a Biotech image scanner (Amersham Pharmacia, Piscataway, NJ). Densitometry was performed using ImageJ software developed at the National Institutes of Health. Calculations for densitometry were: Composition of a given protein subunit, % = [(band or sum of subunit bands)/(all bands measured)] \times 100.

Composition Analysis

The moisture content of the CGM was determined by drying samples at 130°C for 3 hr in a convection oven following Approved Method 44-19.01 (AACC International 2010). The solid content of spent CGM and γ -zein pellet was measured by oven drying overnight at 103°C (Dickey et al 1997). Crude free fat contents were determined by using AACC Approved Method 30-25.01 with the Goldfish apparatus (Labconco, Kansas City, MO) with hexane as solvent. All analyses were conducted in duplicate, and data were expressed on a moisture-free basis. Crude protein contents were determined by using the Dumas nitrogen combustion method with a Vario MAX CN analyzer (Elementar Analyse-systeme GmbH, Hanau, Germany) according to AOAC Method 992.23 (1998). The conversion factor used to estimate protein was $6.25 \times$ nitrogen.

Statistical Analyses

Six different solvents were compared during extractions. Each solvent was run with or without NaOH (0.25%) and the reductant sodium bisulfite (0.5%). The 12 extraction sets were run as a randomized complete block design, replicated twice, and then analyzed using ANOVA and Tukey ($P < 0.05$), for significance using JMP v. 8.0.1 statistical software (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

CGM and Total Zein in CGM

The CGM used for extraction contained 9.2% moisture, 69.6% protein (db), and 2.9% hexane-extractable lipids (db). The particle size distribution of CGM was 3–700 μm . The median particle diameter (d_{50}) was $\approx 100 \mu\text{m}$ and it was used for extraction without modification. The protein contents of CGM fractions including saline-soluble, total zein, and spent CGM were 2, 44, and 21%, respectively. While only a small fraction (2%) of the total protein was saline-soluble protein, a large amount of protein (21%) was insoluble and remained with the spent CGM. The sum of protein percentages measured in three CGM fractions equaled 67%, which compared well to 70% total protein in CGM (db) measured by the Dumas combustion method.

Extraction of Zein Proteins

Solute-to-solvent ratio played a role in zein extraction; preliminary extractions at 1:4 solute-to-solvent ratio with all six solvents were performed as in Carter and Reck (1970) (data not shown). Solvents 70-IPA, 55-IPA, and 70-EtOH extracted total zein proteins; the extracts were highly concentrated and had poor solution stability, similar to zein solutions described in Swallen (1942) and Pomes (1971). This instability caused the protein to gel and precipitate out of solution with spent CGM, decreasing the yield. At

higher solute-to-solvent ratios, the gelling decreased; the zeins were then extracted at the 1:6 solute-to-solvent ratios for all extractions.

The distribution of protein in fractions after the zein extraction, and % protein recovery with different solvents and treatments are presented in Table I. There was no significant difference between the extractions with or without reductant using 88-IPA following Method A; however, 88-IPA extracted significantly less protein than all the solvents following Method B. The solvents that extracted the most protein from CGM as crude zein were 55-IPA, 70-EtOH, and 70-TCP-EtOH with reductant following Method B. These three solvents utilized ethanol or 2-propanol at concentrations that are reported to extract large quantities of zein (Swallen 1942; Pomes 1971), and extracted $\approx 30\%$ more zein protein than 88-IPA. Without the reductant, these three solvents extracted $\approx 20\%$ more protein than 88-IPA, with 55-IPA extracting slightly more protein than the other two solvents. This was because the γ -zein was further reduced and dissolved with the aid of the reductant and low alcohol concentrations. With the steeping step in the corn wet-milling process, many of the disulfide bonds connecting the proteins of the outer layer of the protein body are severed, making the γ -zein extractable even without the benefit of reductant during zein extraction (Esen 1987; Neumann 1987). The presence of reductant during extraction reduced the remaining interconnected γ -zein, thus improving solubility. The sums of the protein contents in crude zein and spent CGM were $\approx 100\%$ for all of the extractions (Table I), except 70-GLY-IPA for extraction with or without reductant. During the measurement of solid content in crude zein from 70-GLY-IPA, there was syneresis of glycerol from the solid matrix. This remaining glycerol could not be homogenized into the sample before protein analysis, thus creating solids containing erroneously higher amounts of protein.

The protein in insoluble zein pellet (thought to be mostly β - and γ -zein, Table I), represented the amount of protein lost in the pellet during purification of α -zeins when the solution concentrations were raised to 88% (w/w) 2-propanol or 95% (v/v) ethanol. For 70-IPA and 55-IPA, the presence of reductant did not significantly change the amount of protein precipitated but there was significantly more protein precipitated with the pellet without the presence of reductant for 70-EtOH and 70-TCP-EtOH. Without the reducing agent in 70-EtOH and 70-TCP-EtOH solvents, a small fraction of α -zein must precipitate out of solution to account for the increase in mass of the insoluble zein pellet. In a similar ex-

traction from CGM using 60% (v/v) 2-propanol containing 1% β -mercaptoethanol, Parris and Dickey (2001) showed that as much as 78% of the insoluble zein pellet was α -zein, 12% γ -zein, and 5% an unknown protein band (14,000 Da). The process by which α -zein precipitates out of solution with γ -zein, even while in a solvent in which α -zein is soluble, is not understood. The solvent 70-GLY-IPA left behind more protein in the insoluble zein pellet, showing that the reducing agent did not have the same effect in reducing the pellet mass in the presence of glycerol.

The protein recoveries for α -zein extracted with 70-IPA, 55-IPA, 70-EtOH, and 70-TCP-EtOH with reductant were the highest with no significant difference between the yields (Table I). However, all four solvents showed a significant difference over the same solvents without reductant. This significantly higher yield for the four solvents with reductant was probably due to the reduction of the remaining interconnected γ -zein, allowing better solubility of all zein. Also, the reductant decreased the amount of protein precipitated in the insoluble zein pellet, increasing the final yield of α -zein due to better extraction and less zein precipitation. Both 88-IPA and 70-GLY-IPA had no difference in yield, regardless of reductant use. For 88-IPA, this probably could be because α -zein is readily extractable without the reducing agent. Small difference in yields were observed for 70-GLY-IPA with or without reductant.

The protein recovery accounted for the portion of protein recovered based on the protein fractions in the initial CGM (Wu et al 1997b), who extracted zein from CGM with a method similar to Method A; their highest protein recovery was 32%. This recovery yield is $\approx 12\%$ less than that obtained with solvents 70-IPA, 50-IPA, 70-EtOH, or 70-TCP-EtOH using Method B in the presence of reductant, and also lesser than for solvents 70-IPA and 55-IPA without reductant. The solvent 70-GLY-IPA extracted the least amount of α -zein regardless of the presence of reductant.

SDS-PAGE and Densitometry

The gel scan of SDS-PAGE of total zein that was extracted following the method of Wu et al (1997b) is presented in Fig. 2. Using the nomenclature of Esen (1987, 1990), total zein contained mostly α -zein with MW of 19,000 and 22,000 Da, γ_2 -zein at 18,000 Da, δ -zein at 10,000 Da, and dimers of α -zein near 45,000 Da. The densitometry scan of the gel indicated that 65% of the fraction was α -zein, 13% γ_2 -zein, 9% δ -zein, and 12% dimers of α -zein. The γ_1 -zein band at MW 28,000 Da was not

TABLE I
Protein Content of CGM Extraction Fractions Extracted with Six Solvents^{a,b}

Treatment	Solvent Systems	Crude Zein ^c (%)	Spent CGM ^d (%)	Insoluble Zein Pellet ^e (%)	Protein Recovery as α -Zein ^f (%)
Reductant and NaOH	88% 2-Propanol	33.4d	69.1a	–	27.9c
	70% 2-Propanol	59.7ab	38.6de	9.0d	44.8a
	55% 2-Propanol	65.0a	33.5f	14.2c	43.2a
	70% 2-Propanol	57.2ab	58.3b	25.0a	9.7d
	w/glycerol				
	70% Ethanol	64.5a	33.7ef	13.7c	44.8a
	70% Ethanol	64.0a	34.7ef	13.2c	43.4a
No Reductant and NaOH	two precipitations				
	88% 2-Propanol	31.3d	70.0a	–	26.7c
	7% 2-Propanol	54.7b	44.2c	10.6d	37.6b
	55% 2-Propanol	57.8ab	40.4cd	13.8c	36.7b
	70% 2-Propanol	44.0c	67.2a	20.7b	7.2d
	w/glycerol				
	70% Ethanol	54.4b	44.0c	20.2b	28.1c
70% Ethanol	54.4b	43.4cd	20.7b	28.5c	
	two precipitations				

^a Values followed by different letters within the same column indicate significant differences ($P < 0.05$).

^b Solvent 88% 2-Propanol followed extraction Method A, the rest of the solvents followed Method B.

^c Crude zein protein = [(mass protein extracted)/(total protein in CGM mass)] \times 100%.

^d Spent CGM protein = [(mass protein unextracted)/(total protein in CGM mass)] \times 100%.

^e Insoluble zein pellet = [(mass protein precipitate in pellet)/(total protein in CGM mass)] \times 100%.

^f Protein recovery = [(protein purity, %) \times (mass α -zein-rich solids)]/(total protein in CGM mass)].

observed in the gel, as reported by Parris and Dickey (2001), who attributed the disappearance of γ_1 -zein to cleavage and loss during the steeping of CGM. Also missing was the β -zein band, which is a protein at 14,000 Da (Wilson 1991).

The molecular bands of α -zein-rich solids obtained using different extraction solvents with or without reductant are presented in Fig. 3. The relative purity of the α -zein-rich solids was analyzed with these SDS-PAGE gels. There was little difference in the α -zein bands with or without the reductant during extraction. The SDS-PAGE of total zein (Fig. 2) showed that it contained proteins that were not observed in α -zein-rich solids extracted (Fig. 3). When considering the protein bands that were in both the total extracted zein (Fig. 2) and α -zein-rich solids (Fig. 3), the amount of extractable α -zein-rich solids was 87% of the total zein

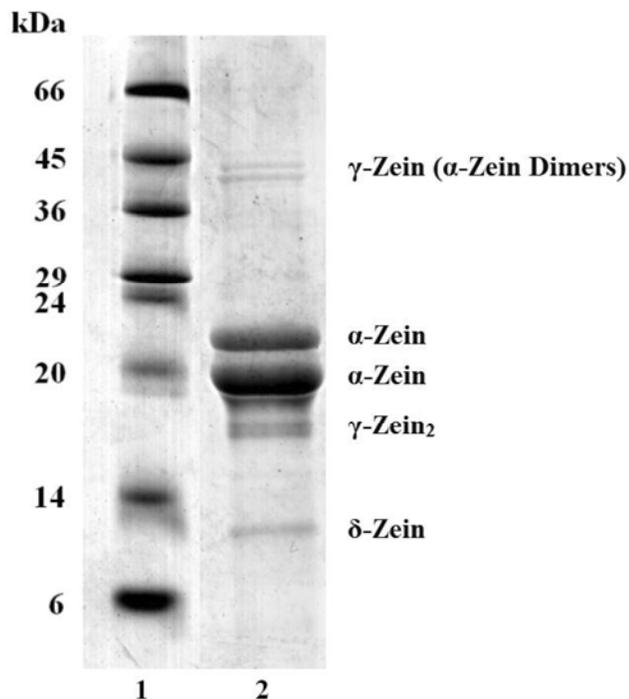


Fig. 2. SDS-Page of total zein extract. Lane 1, marker and lane 2, total zein protein fraction based on the extraction method of Wu et al (1997).

protein extracted. Taking this into account, the α -zein-rich solid yield recovered was 38% of the mass of the CGM. Faint bands at MW 10,000 Da were present in the α -zein-rich solids from solvents 88-IPA, 70-IPA, 55-IPA, 70-EtOH, and 70-TCP-EtOH with and without reductant, which indicated the presence of some δ -zein in such extracts. The extract using 70-GLY-IPA gave α -zein band intensity different than the other five extracts: the 19,000 Da band was less intense, while the 22,000 Da band was more intense. It is not known whether this would affect the properties of the extracted α -zein or whether there were different amounts of minor α -zein proteins in the zein extracted with 70-GLY-IPA.

α -Zein Extraction Efficiency and Yields

The α -zein extraction efficiencies, zein yield, and the protein purities for the solvent systems based on densitometry are presented in Table II. The α -zein extraction efficiencies were \approx 13–79% and was influenced by the solvent makeup and presence of reductant. For a given solvent, α -zein extraction efficiency decreased in the absence of reductant. Prior reduction of zein disulfide bonds during steeping and partial oxidation of disulfide bonds during the drying of CGM (Neumann et al 1987) could be one reason. The γ -zein proteins that are interconnected through disulfide binding must become fully reduced when exposed to the reductant to allow full dissolution of zein (Esen 1987). Extraction with 70-IPA, 55-IPA, 70-EtOH, and 70-TCP-EtOH had α -zein efficiencies \approx 30% higher than 88-IPA with reductant.

The zein yields are included in Table II to compare against literature data; however, the parameter zein yield has been a poor method of reporting yield. The zein yield does not compensate for nonprotein impurities, which could constitute as much as 40–80% of the solid contents in zein extracts (Shukla et al 2000). Bound/trapped moisture is one of the nonprotein parameters that greatly affect zein yield. To determine moisture content in the α -zein-rich solids, they were further ground and dried in a vacuum oven to complete dryness. All samples contained 4–5% moisture indicating that the true protein purities (db) could be higher than values in Table II.

CONCLUSIONS

During the extraction of zein from corn gluten meal, solvents 70-IPA, 55-IPA, and 70-EtOH extracted significantly more α -zein-rich solids using the laboratory-modified extraction method compared to a commercial method. The reductant sodium bisul-

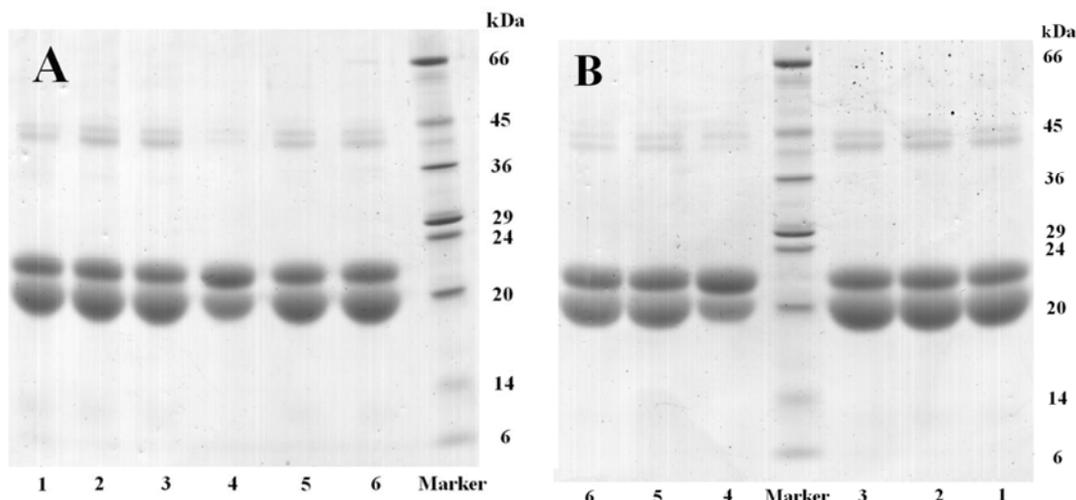


Fig. 3. SDS-PAGE of α -zein extracts. A, Gel of α -zein-rich solids extracted with NaOH and the reductant sodium bisulfite. B, Gel of α -zein fractions were extracted without reductant and NaOH. α -Zein extracted using different solvents: 88% 2-propanol (lane 1), 70% 2-propanol (lane 2), 55% 2-propanol (lane 3), 70% 2-propanol, 22.5% glycerol, and 7.5% water (lane 4), 70% ethanol with one cold precipitation (lane 5), 70% ethanol with two cold precipitations (lane 6).

TABLE II
Zein Yields, Zein Extraction Efficiencies, and Protein Purities^{a,b}

Treatment	Solvent Systems	Protein Purity ^c (%)	α -Zein Extraction Efficiency ^d (%)	Zein Yield ^e (%)
Reductant and NaOH	88% 2-Propanol	83.2c	51.2c	23.4c
	70% 2-Propanol	87.6bc	82.1a	35.6a
	55% 2-Propanol	88.6abc	79.1a	33.9a
	70% 2-Propanol w/glycerol	63.4d	17.8d	10.7d
	70% Ethanol	91.1a-c	82.1a	34.3a
	70% Ethanol	94.0ab	79.6a	32.2ab
	two precipitations			
No Reductant and NaOH	88% 2-Propanol	86.5bc	49.0c	21.5c
	70% 2-Propanol	88.1a-c	68.8b	29.7b
	55% 2-Propanol	88.2a-c	67.3b	29.0b
	70% 2-Propanol w/glycerol	64.5d	13.2d	7.8d
	70% Ethanol	90.7a-c	51.5c	21.6c
	70% Ethanol	96.1a	52.3c	20.7c
	two precipitations			

^a Values followed by different letters within the same column indicate significant differences ($P < 0.05$).

^b Solvent 88% 2-Propanol followed extraction Method A, the rest of the solvents followed Method B.

^c Protein purity is protein content of recovered α -zein-rich solid.

^d α -Zein extraction efficiency = [(Protein purity, %) \times (mass α -zein-rich solids)/(total mass of α -zein protein in CGM)].

^e Zein yield = [(mass α -zein-rich solids)/(total mass of CGM, db)] \times 100%.

fite and NaOH aided in enhancing the zein yields. Dispersal of nonprotein impurities in more dilute aqueous alcohol solvents caused the subsequent cold precipitated α -zein-rich solids to entrap fewer impurities and thus have higher purity. This has implications in eliminating the double-cold-precipitation step in the current process that increases purity of zein proteins. The significance of the research lies in the fact that a modified zein extraction method was devised and shown to extract more zein from CGM substrate. The performance of this modified procedure was evaluated on substrates that vary in quantity and quality of zein present; DDGS from various dry-grind ethanol processes is reported elsewhere.

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LITERATURE CITED

- AACC International. 2010. Approved Methods of Analysis, 11th Ed. Methods 30-25.01 and 44-19.02. Available online only. AACC International: St. Paul, MN.
- AOAC. 1998. Official Methods of Analysis of AOAC International, 16th Ed. 4th rev. Method 992.23. Crude protein in cereal grains and oilseeds—generic combustion method. The Association: Gaithersburg, MD.
- Batterman-Azcona, S. J., and Hamaker, B. R. 1998. Changes occurring in protein body structure and α -zein during cornflake processing. *Cereal Chem.* 75:217-221.
- Carter, R., and Reck, D. R. 1970. Low temperature solvent extraction process for producing high purity zein. US patent 3,535,305.
- Coleman, R. E. 1939. Zein solution and coating composition. US patent 2,185,111.
- Coleman, R. E. 1941. Ink. US patent 2,236,521.
- Cook, R. B., Mallee, F. M., and Shulman, M. L. 1996. Purification of zein from corn gluten meal. US patent 5,580,959.
- Cox, M. J., MacMasters, M. M., and Hilbert, G. E. 1944. Effect of the sulfurous acid steep in corn wet milling. *Cereal Chem.* 21:447-465.
- Dickey, L. C., Dallner, M. F., Radewonuk, E. R., Parris, N., Kurantz, M., and Craig, J. C. 1997. Hydrocyclone separation of dry-milled corn. *Cereal Chem.* 74:676-680.
- Dong, J., Sun, Q., and Wang, J.-Y. 2004. Basic study of corn protein, zein, as a biomaterial intissue engineering, surface morphology and biocompatibility. *Biomaterials* 25:4691-4697.
- Esen, A. 1986. Separation of alcohol-soluble proteins (zeins) from maize into three fractions by differential solubility. *Plant Physiol.* 80:623-627.
- Esen, A. 1987. A proposed nomenclature for the alcohol-soluble proteins (zeins) of maize (*zea-mays*-1). *J. Cereal Sci.* 5:117-128.
- Esen, A. 1990. An immunodominant site of gamma-zein1 is in the region of tandem hexapeptide repeats. *J. Protein Chem.* 9:453-460.
- Gorham, J. 1821. Analysis of Indian corn. *Q. J. Sci. Lit. Art.* 2:206-208.
- Jiang, Q., Reddy, N., and Yang, Y. 2010. Cytocompatible cross-linking of electrospun zein fibers for the development of water-stable tissue engineering scaffolds. *Acta Biomaterials* 6:4042-4051.
- Kale, A., Zhu, F., and Cheryan, M. 2007. Separation of high-value products from ethanol extracts of corn by chromatography. *Ind. Crops Prod.* 26:44-53.
- Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of head of bacteriophage-t4. *Nature* 227:680-685.
- Landry, J., Paulis, J. W., and Fey, D. A. 1983. Relationship between alcohol-soluble proteins extracted from maize endosperm by different methods. *J. Agric. Food Chem.* 31:1317-1322.
- Lougovoy, B. N. 1949. Chewing gum base. US patent 2,489,147.
- Mohammad, K. B., and Esen, A. 1990. Zein degradation in the endosperm of maize seeds during germination. *Am. J. Bot.* 77:973-980.
- Neumann, P. E., Wall, J. S., and Walker, C. E. 1984. Chemical and physical-properties of proteins in wet-milled corn gluten. *Cereal Chem.* 61:353-356.
- Osborne, T. 1891. Process of extracting zein. US patent 456,773.
- Osborne, T. B., Mendel, L. B., Ferry, E. L., and Wakeman, A. J. 1914. Nutritive properties of proteins of the maize kernel. *J. Biol. Chem.* 18:1-16.
- Parris, N., and Dickey, L. C. 2001. Extraction and solubility characteristics of zein proteins from dry-milled corn. *J. Agric. Food Chem.* 49:3757-3760.
- Pomes, A. F. 1971. Zein. Pages 125-132 in: *Encyclopedia of Polymer Science and Technology*, Vol 15. H. F. Mark and N. G. Gaylord, eds. John Wiley and Sons: New York.
- Shukla, R., and Cheryan, M. 2001. Zein: The industrial protein from corn. *Ind. Crops Prod.* 13:171-192.
- Shukla, R., Cheryan, M., and DeVor, R. E. 2000. Solvent extraction of zein from dry-milled corn. *Cereal Chem.* 77:724-730.
- Simonds, H. R., Weith, A. J., and Bigelow, M. H. 1949. Manufacture of zein plastic. Pages 754-756 in: *Handbook of Plastics*, 2nd Ed. Van Nostrand: New York.
- Sturken, O. 1938. Composition of matter. US patent 2,115,240.
- Swallen, L. C. 1942. Process for the production of zein. US patent 2,287,649.
- Takahashi, H., and Yanai, N. 1994. Process for refining zein. US patent 5,342,923.
- Tu, J., Wang, H., Li, H., Kerong, D., Zhang, X., and Wang, J. 2009. The in vivo bone formation by mesenchymal stem cells in zein scaffolds. *Biomaterials* 26:4369-4376.
- Wang, H.-J., Gong, S.-J., Lin, Z.-X., Fu, J.-X., Xue, S.-T., Huang, J.-C., and Wang, J.-Y. 2007. In vivo biocompatibility and mechanical proper-

ties of porous zein scaffolds. *Biomaterials* 28:3952-3964.
Wilson, C. M. 1991. Multiple zeins from maize endosperms characterized by reversed-phase high-performance liquid-chromatography. *Plant Physiol.* 95:777-786.
Wu, S., Myers, D. J., Johnson, L. A., Fox, S. R., and Singh, S. K. 1997a.

Pilot-plant wet-milling process for producing corn gluten meal. *Cereal Chem.* 74:264-267.
Wu, S., Myers, D. J., and Johnson, L. A. 1997b. Factors affecting yield and composition of zein extracted from commercial corn gluten meal. *Cereal Chem.* 74:258-263.

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