

Effects of Maize Hybrid and Meal Drying Conditions on Yield and Quality of Extracted Zein¹

SHAOWEN WU,² DELAND J. MYERS,^{2,3} and LAWRENCE A. JOHNSON^{2,4}

ABSTRACT

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Corn gluten meal (CGM) produced from two maize hybrids and subjected to five drying treatments were used to determine the effects of variables on zein extraction. Zein extraction yields, protein recoveries, and purities were higher in the CGM with the higher protein content. The yield and protein recovery of zein decreased as the drying temperature increased. Zein yield, protein recovery, and purity were significantly

lower in the CGM subjected to freeze- and spray-drying than in those subjected to oven-drying. A relatively higher pH value in the CGM slurries was a characteristic of freeze- and spray-dried CGM. An explanation of these results based on the mechanisms of protein changes during drying is given.

Zein has been commercially extracted from corn gluten meal (CGM), a coproduct of wet milling, since the 1940s (Swallen 1942). The current commercial process, based on the patent of Carter and Reck (1970), uses a hot isopropyl alcohol-alkaline solution to extract zein and then chills the extract to separate the zein from the solvent. High variation in yield and quality plagues processors. Although there have been numerous studies on the technology of zein extraction, such as alternative extraction solvents, changes in temperature, extraction time, and ratio of solvent to CGM (Swallen 1941, Evans et al 1945, Russell and Tsao 1982), studies investigating how CGM quality affects zein extraction yield and quality have not been published.

Watson and Yahl (1967) showed that the maize hybrid used as well as the severity of artificial drying affect milling results, including CGM yield and quality. Extensive heat treatment may also cause chemical and physical changes to proteins and decrease extractability. McGuire and Earle (1958) reported that the proteins extracted with water, 5% salt solution, and 0.01*N* KOH solution decreased significantly ($P < 0.05$) when the drying temperature of the corn kernels increased from 48.9 to 93.3°C. The nitrogen content of extracts obtained with 60% ethanol at 79.4°C was no different from that of an air-dried sample or a sample dried at 93.3°C. There was also no indication of critical damage occurring at any particular temperature (ambient to 93.3°C) during corn drying. Watson and Hirata (1962) observed that the soluble-protein contents of steepwater filtrate and ground-steeped grain decreased when the drying temperature of corn kernels increased from 60 to 93.3°C. Wall and coworkers (1975) reported that a substantial decrease in salt-soluble proteins and a small decrease in alcohol-soluble proteins occurred when whole corn was dried from 25 to 15% moisture at 143°C. They also observed decreased sulfhydryl content with increasing temperature. They indicated that extensive heat treatment of native whole corn denatured protein and caused molecular aggregation through noncovalent hydrophobic interaction and covalent disulfide cross-links contributing to protein insolubility. Weller et al (1987) observed that ethanol-soluble protein

decreased severely when the high harvest moisture corn (30% moisture) was dried from 50 to 71°C. They hypothesized that the solubility loss was most likely due to chain unfolding and the formation of new intermolecular disulfide bonds within endosperm protein.

Neumann and coworkers (1984) compared the proteins extracted from commercial wet and dried CGM. They found that the yields of alcohol-soluble proteins were higher from CGM than from native corn. They indicated that SO₂ added in the steepwater cleaved protein disulfide bonds of corn proteins during steeping and increased zein extractability. Wet CGM contained more salt-soluble protein than did dried CGM, but the amount of alcohol-soluble protein was only slightly higher in wet than in dried CGM. About 50% of cysteine-cystine in the commercial CGM was present in sulfhydryl or disulfide form (Neumann et al 1984). The remaining 50% was in the S-sulfocysteine form, which is stable at neutral and mildly acidic pH and does not readily decompose during commercial drying. About 5–10% of the total cysteine-cystine content was present as cysteine in the wet CGM, but no cysteine was found in commercially dried CGM. This indicated that the absence of cysteine and the presence of a large amount of cystine in the dried CGM must be due to the quantitative oxidation of sulfhydryls to disulfide bonds during heating.

The objectives of the present study were to: 1) determine the influence of drying temperature and drying method on the yield and purity of extracted zein; 2) investigate the effects of the maize hybrid on the zein yield and purity; and 3) evaluate the changes of proteins in the CGM after wet milling and drying.

MATERIALS AND METHODS

Preparation of CGM and Corn Endosperm Meal

CGM was produced from two maize hybrids, Pioneer 3394 (low protein content variety) and Wilson D110 (high protein content variety) using a pilot-plant wet-milling process (Wu et al 1997a). Both maize hybrids grew in the same location in Iowa, were harvested in 1994, and dried, stored, and milled in the same conditions. The CGM was dried by three different methods: oven, freeze, and spray. The forced-air oven drying was done at 50, 100, and 150°C. The spray-drying was performed with a mini-spray dryer (Yamato Scientific Co., Japan) with an inlet temperature of 120°C and outlet temperature of 60°C. Pioneer 3394 CGM contained 49.8% protein and 11% crude fat. Wilson D110 CGM had 53.7% protein and 7.0% crude fat (Wu et al 1997a). All CGM samples were stored at 4°C.

Corn endosperm meal was produced by first soaking corn kernels in deionized water at 50°C overnight. After the pericarp and

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²Graduate research assistant, associate professor and professor, respectively.

³Corresponding author. E-mail: dmyers@iastate.edu

⁴Professor-in-charge, Center for Crops Utilization Research, Iowa State University, Ames, IA 50011.

germ were removed by hand, the softened endosperm was ground with a mortar and pestle. The endosperm meal was dried at room temperature in a hood, passed through a 35-mesh screen, and stored in a refrigerator. The moisture content of the endosperm meal was measured by the Karl Fischer method (model 392, Fisher Scientific, Pittsburgh, PA) (ASTM 1975).

Zein Extraction

Zein was extracted from CGM using 88% hot isopropanol at pH 12.5, and separated by subsequent chilling (Carter and Reck 1970, Wu et al 1997b), except that the ratio of CGM to solvent was changed from 1:4 to 1:6. Zein extraction yield was calculated as the percentage of the initial CGM weight on a dry basis (db). Protein recovery of zein extraction was calculated as the ratio of the protein content in the extracted zein to the protein content in the CGM (db). Protein purity was calculated as the protein content in the extracted zein. The Kjeldahl (Tecator, Sweden) method (CRA 1986) was used to measure total nitrogen content ($N \times 6.25$).

Separation of Protein Fractions

The proteins in CGM samples (0.40 g) and corn endosperm powder (2.0 g) were separated into the salt-aqueous soluble protein, alcohol-soluble protein with reducing reagent, and alcohol-insoluble fractions. Samples were extracted with 0.5M NaCl solution for 20 min at room temperature (10 mL for CGM, 20 mL for corn endosperm powder) (Osborne and Mendel 1914). After centrifugation for 15 min at $30,000 \times g$ (model J2-21, Beckman, Palo Alto, CA), the supernatant was collected. The extraction was repeated once, and the supernatants were combined for protein analysis. The residues were extracted with 55% (v/v) isopropyl alcohol and 5% (v/v) 2-mercaptoethanol plus 0.5% (w/v) sodium acetate (PMA) (10 mL for CGM, 20 mL for corn endosperm powder) (Wilson 1991). The mixture was shaken for 2 hr at room temperature, 130 rpm, and centrifuged at $30,000 \times g$ for 10 min. The supernatant was collected, and the extraction with PMA was repeated once. The residue was then washed with PMA (5 mL for CGM, 10 mL for corn endosperm powder) and centrifuged. All extracted supernatants and wash solutions were collected and analyzed for protein content. Protein content for each fraction was measured by using the Kjeldahl method for nitrogen content ($N \times 6.25$).

TABLE I
Zein Extraction Yield^a of Corn Gluten Meal Dried Under Different Conditions^b

Hybrid	Drying Treatment				
	Oven				
	50°C	100°C	150°C	Freeze	Spray
Wilson D110	21.5aA	18.2bA	17.1bcA	16.4cA	16.4cA
Pioneer 3394	17.8aB	17.2aB	16.8aA	10.4bB	11.5cB

^a % of initial weight, db.

^b Means of nine replicates. Data in the same row with different lower case letters were significantly different at $P < 0.05$. Data in the same column with different capital letters were significantly different at $P < 0.05$.

TABLE II
Protein Recovery (%) of Zein Extracted from Corn Gluten Meal Dried Under Different Conditions^a

Hybrid	Drying Treatment				
	Oven				
	50°C	100°C	150°C	Freeze	Spray
Wilson D110	32.1aA	26.0bA	24.1 bA	24.4bA	24.8bA
Pioneer 3394	27.5aB	25.7bA	24.2bA	14.2cB	15.7cB

^a Means of nine replicates. Data in the same row with different lower case letters were significantly different at $P < 0.05$. Data in the same column with different capital letters were significantly different at $P < 0.05$.

HPLC Analysis

Samples for high performance liquid chromatography (HPLC) analysis were prepared by extracting corn endosperm powder (1.25 g) and CGM (0.30 g) with 5 mL of PMA for 2 hr at room temperature. The supernatant was diluted 10 times with 55% (v/v) isopropyl alcohol and 5% (v/v) 2-mercaptoethanol (PM) solvent before injection. Zein (30 mg) was dissolved in 1 mL of PMA and diluted 10 times before injection.

The reverse-phase HPLC analysis equipment included an HP-1050 HPLC system with an automatic sample injector, gradient solvent delivery system, oven heater, a diode array detector, and a 486 computer with a Chemstation program. A Vydac (Hesperia, CA) C₁₈ column (25 cm \times 4.6 mm, 5- μ m particle size, 300 Å pore size) was used to analyze the samples (Dombrink-Kurtzman and Bietz 1993). Zein separation was performed by using nonlinear gradients of increasing acetonitrile (ACN) in water with 0.1% (v/v) trifluoroacetic acid (TFA). The starting buffer was 30% ACN, increasing to 50% ACN at 20 min (at 1.0%/min), then to 56% ACN after 35 min (0.17%/min), and ending at 64% ACN after 5 min (5.8%/min). The column was operated at 55°C and a flow rate of 1.0 mL/min. A 20- μ L sample (\approx 20–30 μ g of protein) was injected for analysis. The eluate was monitored at 214 and 280 nm on the diode array detector.

Protein Deamidation and NH₃ Determination

Deamidation of CGM protein was conducted in a 25- \times 150-mm test tube fitted with a rubber stopper carrying two glass tubes. One glass tube was inserted into the test tube \approx 2 cm from the bottom and connected to a high purity helium tank (99.99%). The other glass tube was connected to a collection tube. Before the reaction, the CGM sample (1 g) was thoroughly mixed with a preweighed amount of deionized distilled water to reach 30% (w/w) moisture content. The reaction tube was then heated in a bath at a constant temperature of 110°C for 2.5 hr. Helium gas (100 mL/min) was passed through the reaction tube during heating and carried released NH₃ to the collection tube containing 10 mL of 1N HCl solution. Aliquots (5 mL) of HCl were diluted to 10 mL with deionized distilled water before analysis with an ammonia electrode (Orion Research Inc., Boston, MA). A reference curve was prepared using standard ammonium chloride solutions with a range of 10 to 10⁻² ppm. Deamidation was replicated three times for each CGM sample.

pH of CGM Slurry

The pH of the CGM slurry was determined with an ionalyzer (model 501, Orion Research Inc., Boston, MA) after stirring 10 g of CGM in 20 mL of deionized water for 10 min. The deionized water was boiled to remove CO₂ before analysis.

Statistical Analysis

Statistical analysis used a randomized complete block design. Three millings were three blocks. CGM drying treatments were a factorial combination of hybrid and treatment (2 \times 5). The general linear model and the test of least significant difference (LSD) at the 5% level were used to determine the significant difference between means.

RESULTS AND DISCUSSION

Drying Temperature Effect on Zein Extraction Yields

Zein extraction yields from CGM dried in a forced-air oven at different temperatures were 17.1–21.5% for Wilson D110 and 16.8–17.8% for Pioneer 3394 (Table I). The yield was significantly higher for the Wilson D110 CGM dried at 50°C than for those dried at 100 and 150°C. The zein extraction yield of Pioneer 3394 also decreased as the oven-drying temperature increased, although the changes were not significantly different at $P < 0.05$. The higher zein yield obtained at 50°C indicates that protein was less aggregated during drying.

Zein extraction yield was affected by the genotype of the maize. The yields of zein extracted from Wilson D110 were higher than those from Pioneer 3394 at the same temperatures, although there were no significant differences between the yields extracted from the CGM dried at 150°C. The results also show that there was an interaction between hybrids and drying temperature. High temperature treatment not only decreased zein extractability but also decreased the difference caused by maize genotype.

Drying Temperature Effect on Protein Recoveries

Protein recoveries showed trends similar to yield (Table II). Protein recovery from CGM dried at 50°C was significantly greater than that from CGM dried at 100 and 150°C for both hybrids. There were interactions between hybrids and oven-drying treatments for protein recovery. The hybrid influence on protein recovery was significantly different at 50°C but not at higher drying temperatures.

Protein fraction analysis showed that hybrid means were significantly different in total protein, extractable albumin and globulin, and zein contents in oven-dried CGM (Table III). The oven-dried CGM of Wilson D110 had higher total protein, extractable albumin and globulin, and zein content than the CGM of Pioneer 3394. The higher original extractable zein content in the CGM can account for the higher extraction yield and protein recovery. There were no significant differences for the total protein, and albumin and globulin contents at different oven-drying temperatures; however, the zein content of CGM dried at 150°C was significantly lower than that of the CGM dried at 50 and 100°C. The lower zein content in the CGM dried at 150°C may be due to zein aggregation during drying, formation of large insoluble polymers that contribute to zein insolubility. Therefore, the extraction yields and protein recoveries were the lowest in the CGM dried at 150°C. The interaction effects of maize hybrid and temperature may indicate that the zein aggregation in the high-

protein content CGM is more sensitive to temperature change than the zein in the low-protein content CGM.

Drying Temperature Effect on Protein Purities

Protein purity of the extracted zein significantly decreased as the drying temperature increased from 50 to 150°C ($P < 0.05$) (Table IV), and the mean of 50°C treatment exceeds the means of the 100 and 150°C treatments by 3.1 and 5.2%, respectively. Wilson D110 had a significantly greater protein purity than that of Pioneer 3394 ($P < 0.05$), and the mean difference between the hybrids was 8.1% in oven drying. The low purity may be explained by the presence of other alcohol-soluble compounds, such as oil and pigments, in the CGM. The CGM of Pioneer 3394 contained more oil than did the CGM of Wilson D110 (Wu et al 1995a), which could explain why the protein purity values for Pioneer 3394 were low. Zein binding with other components such as oil (Izzo and Ho 1989) or products of the Maillard reaction at high temperature may be another reason.

α -Zein Content and Recovery

HPLC analysis of α -, β -, and γ -zeins in corn endosperm meal and CGM indicated that wet milling decreased measured amounts of β - and γ -zein (Figs. 1 and 2). The β - and γ -zein content of Wilson D110 endosperm meal was 3.34 and 19.73%, respectively, of the total zein content, and the values decreased to 0.83 and 5.67%, respectively, of the total zein content in the wet gluten. The β - and γ -zein content of Pioneer 3394 also decreased from 5.49 and 21.25% in the endosperm meal to 1.72 and 10.41%, respectively, in the wet gluten. Hybrid variation not only causes variation in α -zein component distributions, but also affects α -, β -, and γ -zein contents. Because the calculations of α -, β -, and γ -zeins were on a percentage basis, Wilson D110 had lower extractable β - and γ -zein content than Pioneer 3394 and, therefore, a higher α -zein content than Pioneer 3394 (Table V). Drying temperature did not

TABLE III
Protein Composition (db) in Corn Gluten Meal (CGM) Dried Under Different Conditions^a

Components	Drying Treatment					
	Oven			Freeze	Spray	Hybrid Means ^b
	50°C	100°C	150°C			
CGM protein (%)						
Wilson D110	56.6 ± 1.3	56.5 ± 1.2	56.5 ± 1.0	54.3 ± 1.1	53.9 ± 2.6	55.6a
Pioneer 3394	49.1 ± 2.8	49.2 ± 3.3	49.1 ± 2.9	49.1 ± 2.0	48.6 ± 3.3	49.0b
Treatment means ^c	52.8a	52.9a	52.8a	51.7a	51.3a	...
Extractable Alb + Glo (%)						
Wilson D110	1.3 ± 0.2	1.2 ± 0.3	1.4 ± 0.2	2.3 ± 0.5	1.7 ± 0.4	1.6a
Pioneer 3394	0.8 ± 0.2	0.8 ± 0.1	0.7 ± 0.2	1.9 ± 0.1	1.6 ± 0.6	1.2b
Treatment means ^c	1.1a	1.0a	1.1a	2.1b	1.7c	...
Extractable zein (%)						
Wilson D110	37.8 ± 0.9	37.4 ± 0.9	34.5 ± 0.8	37.3 ± 1.0	37.4 ± 0.5	36.9a
Pioneer 3394	29.5 ± 1.5	29.3 ± 1.4	26.9 ± 0.4	28.5 ± 0.8	28.9 ± 1.0	28.7b
Treatment means ^c	33.7a	33.4a	30.7b	32.9a	33.3a	...

^a Means of six replicates.

^b Data in the same column with different letters were significantly different at $P < 0.05$.

^c Data in the same row with different letters were significantly different at $P < 0.05$.

TABLE IV
Protein Purity (%) of Zein Extracted from Corn Gluten Meal (CGM) Dried Under Different Conditions^a

Hybrid	Drying Treatment					
	Oven			Freeze	Spray	Hybrid Means ^b
	50°C	100°C	150°C			
Wilson D110	84.4	80.5	79.5	80.4	81.5	81.3a
Pioneer 3394	76.0	73.7	70.6	66.7	66.1	70.6b
Treatment means ^c	80.2a	77.1b	75.0c	73.5c	73.8c	...

^a Means of nine replicates.

^b Data in the same column with different letters were significantly different at $P < 0.05$.

^c Data in the same row with different letters were significantly different at $P < 0.05$.

significantly affect the extractable β - and γ -zein content, but the extractable α -zein content was significantly different in the CGM dried at 50 and 150°C (Table V). Because the process is designed for α -zein extraction (Carter and Reck 1970), the relatively low total extractable α -zeins would produce low extraction yield and protein recovery.

In an ideal situation, the amount of extracted protein should equal the extractable α -zein content in the CGM, i.e., 100% α -zein recovery. The α -zein recoveries for Wilson D110 CGM were 51.3, 42.0, and 42.3% for the 50, 100, and 150°C treatments, respectively. The α -zein recoveries for Pioneer 3394 CGM were 52.3, 49.5, and 50.5% for the 50, 100, and 150°C treatments, respectively. The low α -zein recovery values may result from the formation of cross-linking disulfide bonds in some α -zeins during heating. The amount of SO₂ used in the wet-milling steeping process should have cleaved the disulfide bonds among the zeins and converted them to cysteine and S-sulfocysteine residues. The S-sulfocysteine residues are stable in neutral and mildly acidic conditions, with no degradation in commercial drying conditions (Neumann et al 1984). However, the cysteine residues can reform disulfide bonds in the presence of oxygen during heating. Wall et al (1975) observed a significant decrease in sulfhydryl content in corn when the temperature increased from 15 to 143°C. Neumann et al (1984) also reported that CGM proteins formed disulfide bonds during commercial drying. Therefore, as drying temperature increases, more disulfide bonds reform. The α -zeins with S-sulfocysteine residues can be extracted as monomers or oligomers by an aqueous alcohol, but cross-linked zeins cannot be easily extracted. Because Wilson D110 CGM contained more protein than Pioneer 3394 CGM, the same amount of SO₂ or steeping time may not be sufficient to cleave all the disulfide bonds. Therefore, the α -zein recovery values of Wilson D110 CGM were lower than those of Pioneer 3394. The major oxidation reaction of cysteine residues seems to occur at 50–100°C because the α -zein recovery values decreased significantly in this temperature range.

Effect of Freeze- and Spray-Drying on Zein Yield, Protein Recovery, and Purity

When drying methods for Wilson D110 CGM were compared, the yield, protein recovery, and protein purity of zein extracted from freeze- and spray-dried CGM had similar values that were not significantly different from the values of oven drying at 150°C, but were much lower than those oven dried at 50°C ($P < 0.05$) (Tables I, II, and IV). Zein extracted from Pioneer 3394 CGM had a similar trend, but extraction yields and protein recoveries from freeze- and spray-dried CGM were much lower than those of all the oven-drying treatments. Hybrid effects were sig-

nificant ($P < 0.05$) on yield, protein recovery, and protein purity in the freeze- and spray-drying treatments. Drying method influenced zein extraction more for Pioneer 3394 than for Wilson D110.

Protein Composition in Freeze- and Spray-Dried CGM

Total protein content of the freeze- and spray-dried CGM was slightly lower but not significantly different from that of oven-dried CGM (Table III). The content of extractable albumin and globulin, however, was about 60% greater in the spray-dried and approximately twice as great in the freeze-dried CGM than in the oven-dried CGM. One reason for the greater amounts of water- and salt-soluble proteins in the freeze- and spray-dried CGM was the difference in the collection of the CGM before drying. Gluten used for oven drying was dewatered with a vacuum drum filter, whereas the gluten used for freeze- and spray-drying treatments was concentrated by siphoning free liquid after settling. The solids content in the wet gluten for oven drying was 40–46%; it was only 1.8–2.0% in the gluten slurry used for freeze- and spray-drying. Therefore, the CGM dried from the gluten slurry contained more water-soluble compounds, including water- and salt-soluble peptides and proteins, sugars, and organic acids. A more important reason for the high amount of water- and salt-soluble proteins may be that there was less oxidation of cysteine in the freeze- and spray-drying processes. Less disulfide bond formation among proteins will markedly increase the extractability of the water- and salt-soluble proteins. The zein content of the freeze- and spray-dried CGM was not significantly different from that of oven-dried CGM at 50 and 100°C.

The relative ratios of α -, β -, and γ -zeins in the freeze- and spray-dried CGM were significantly different from those in oven-dried CGM (Table V). The β - and γ -zein contents were higher in the freeze- and spray-dried CGM than those in the oven-dried CGM. Because of the high cysteine content of the γ - and β -zeins (Shewry and Tatham 1990), as temperature increases, γ - and β -zeins can form disulfide bonds more easily than can α -zeins. Reduced heat treatment and shorter oxygen exposure time gave less opportunity for disulfide bond formation in β - and γ -zeins. Therefore, the freeze- and spray-dried CGM contained a relatively high content of β - and γ -zeins and a low content of α -zeins.

α -Zein Recoveries in Freeze- and Spray-Dried CGM

The α -zein recovery values were 38.4 and 38.7% for Wilson D110 CGM for the freeze- and spray-drying treatments, respectively, and 28.2 and 30.4% for Pioneer 3394 for the freeze- and spray-drying treatments, respectively. The α -zein recoveries of the freeze- and spray-drying treatments were much lower than that of the oven-drying treatment and even lower than that of the 150°C

TABLE V
Extractable Zein Composition (%) in Corn Gluten Meal Dried Under Different Conditions^a

Components	Drying Treatment					
	Oven			Freeze	Spray	Hybrid Means ^b
	50°C	100°C	150°C			
α -Zein (%)						
Wilson D110	93.6 ± 0.2	93.3 ± 0.2	93.2 ± 0.3	92.2 ± 0.3	92.3 ± 0.3	92.9a
Pioneer 3394	87.7 ± 0.3	87.5 ± 0.2	87.3 ± 0.1	86.4 ± 0.1	86.6 ± 0.3	87.1b
Treatment means ^c	90.6a	90.4ab	90.2b	89.3c	89.5c	
β -Zein (%)						
Wilson D110	1.07 ± 0.02	1.07 ± 0.01	1.12 ± 0.03	1.12 ± 0.03	1.18 ± 0.01	1.11a
Pioneer 3394	2.49 ± 0.34	2.59 ± 0.26	2.65 ± 0.38	2.91 ± 0.31	2.77 ± 0.35	2.68b
Treatment means ^c	1.78a	1.83ab	1.89ab	2.02b	1.98ab	
γ -Zein (%)						
Wilson D110	5.35 ± 0.21	5.66 ± 0.23	5.71 ± 0.27	6.64 ± 0.37	6.48 ± 0.34	5.97a
Pioneer 3394	9.80 ± 0.13	9.96 ± 0.29	10.03 ± 0.35	10.65 ± 0.34	10.61 ± 0.41	10.21b
Treatment means ^c	7.58a	7.81a	7.87a	8.64b	8.55b	

^a Means of six replicates.

^b Data in the same column with different letters were significantly different at $P < 0.05$.

^c Data in the same row with different letters were significantly different at $P < 0.05$.

treatment. The extractable α -zein content, however, was not significantly lower in the freeze- and spray-dried CGM than in the oven-dried CGM treated at 50 and 100°C. Extractable α -zein content was also significantly higher than that of 150°C oven treatment (Table III).

Low values for yield, protein recovery, and α -zein recovery are theoretically the result of the unavailability of some α -zeins in the freeze- and spray-dried CGM. Wall and coworkers (1975) indicated that when protein denatures, the noncovalent hydrophobic interactions and covalent disulfide bonds that cause molecular aggregation in zein may be broken. Because there was less heat in the freeze- and spray-drying processes, denaturation may not have occurred, resulting in less α -zein available for extraction. This, in effect, could have resulted in relatively higher amounts of extractable albumin, globulin, β -, and γ -zein in the freeze- and spray-dried CGM.

Zein Deamidation During Drying

Because α -zein contains $\approx 20\%$ glutamic acid and glutamine residues, and because 90% of this residue is present as glutamine (Righetti et al 1977), α -zein deamidation during oven heating is possible. Zhang et al (1993a) measured the percentage of thermal deamidation in soy protein, casein, lysozyme, and gliadin when the proteins were heated at 115°C for 2 hr in a water-limited environment. They reported that gliadin, unlike other proteins, had a maximum deamidation ($\approx 8\%$) at a moisture content of $<10\%$ because it contains $\approx 30\%$ glutamine. The deamidation at acidic conditions (pH 3.0) was probably by a direct hydrolysis pathway, and the primary protein sequence did not affect the deamidation rate (Zhang et al 1993b). Deamidation was accelerated by increased pH and the presence of anions, such as phosphate, bicarbonate (Shih 1990), chlorate, and sulfate (Shih and Kalmar 1987). The ammonia released from the amide could play an important role in the nonenzymatic browning reaction. More available amide in proteins results in more Maillard reaction (Izzo and Ho 1993).

Deamidation in acidic conditions removes amides from protein, generates acidic side chains, and increases the charge density on proteins. Protein conformation also changes during deamidation by increasing electrostatic repulsion and decreasing hydrogen bonding (Matsudomi et al 1982). Changes of charge density and protein conformation led to protein unfolding and, thus, enhanced protein solubility (Matsudomi et al 1981). Deamidation of zein increased solubility (Casella and Whitaker 1990). Because more heat was used in oven-dried CGM, more deamidation of protein could occur in the oven-dried CGM. Therefore, more zein in the oven-dried CGM could dissolve in the isopropanol-water extraction solvent, and extraction yield and protein recovery were higher from oven-dried CGM than from freeze- and spray-dried CGM.

The results reported in Table VI on the amounts of ammonia released from deamidation of CGM protein support our hypothesis. That the greatest amount of ammonia was released from freeze-dried CGM indicates that the lowest level of deamidation occurred during freeze-drying. The $\text{NH}_3/\text{protein}$ value obtained from spray-dried CGM was significantly lower than that of freeze-dried, but significantly higher than that of oven-dried. The lowest $\text{NH}_3/\text{protein}$ value obtained from the oven-dried CGM samples at 50°C also demonstrated that temperature and heat-treating CGM for an extended period of time caused more deamidation of glutamine (almost four times more) than did freeze-drying. Both hybrids had similar trends of deamidation for the different drying treatments.

The pH of the CGM slurry slightly increased as the oven temperature increased from 50 to 150°C (Table VII). The change was greater in the Wilson D110 hybrid than in the Pioneer 3394 hybrid. The pH values of freeze- and spray-dried CGM slurries were similar and significantly higher than the pH values of oven-dried CGM slurries. This difference in CGM slurry pH could have been caused by protein changes during drying. Disulfide bond formation will increase the slurry pH value, and deamidation of glutamine during heating will decrease the slurry pH value.

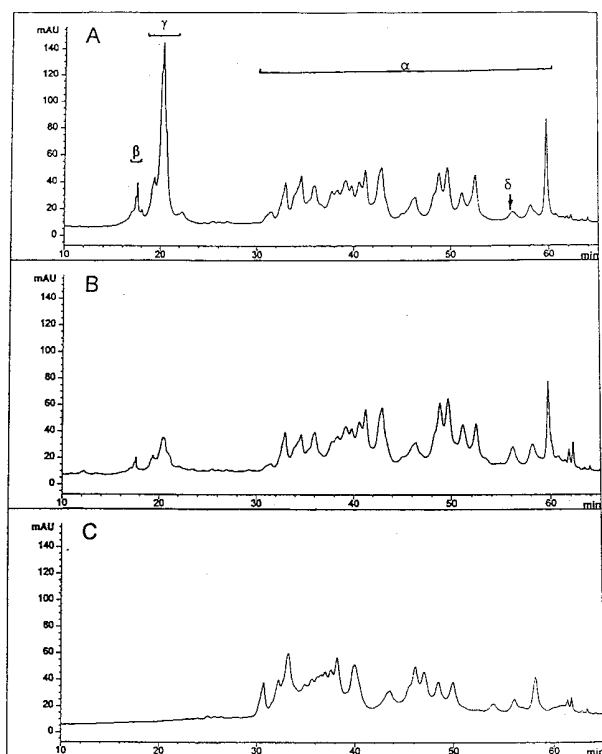


Fig. 1. Reversed-phase high-performance liquid chromatography (RP-HPLC) analysis of alcohol-soluble proteins from Wilson D110 corn (A), corn gluten meal (B), and extracted zein (C). α , β , γ , and δ = zein classes.

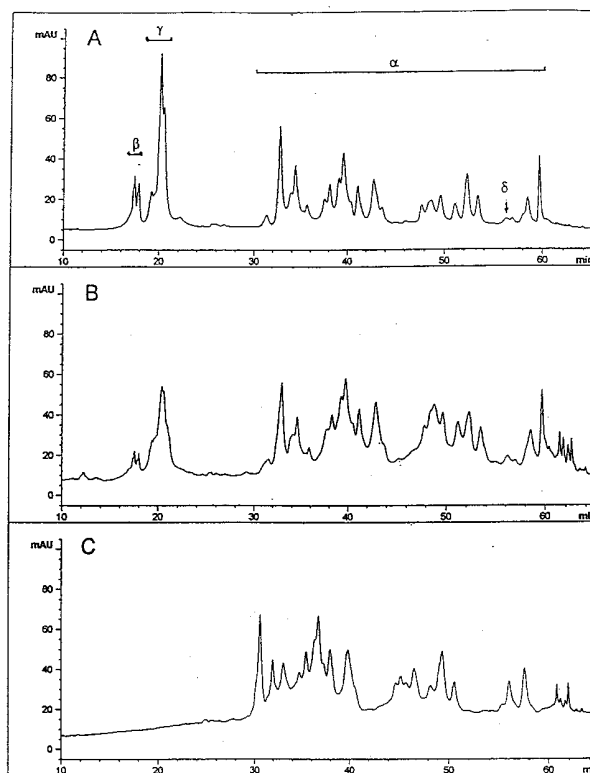


Fig. 2. Reversed-phase high-performance liquid chromatography (RP-HPLC) analysis of alcohol-soluble proteins from hybrid Pioneer 3394 corn (A), corn gluten meal (B), and extracted zein (C). α , β , γ , and δ = zein classes.

TABLE VI
Ammonia Released in Deamidation^a

Corn Gluten Meal	NH ₃ /Protein (mg/g)
Wilson D110	
Oven drying	
50°C	0.0031e
100°C	0.0042d
150°C	0.0041d
Freeze drying	0.0157a
Spray drying	0.0062c
Pioneer 3394	
Oven drying	
50°C	0.0036d,e
100°C	0.0048d
150°C	0.0048d
Freeze drying	0.0125b
Spray drying	0.0060c

^a Means of three replicates. Data in the same row with different letters were significantly different at $P < 0.05$.

TABLE VII
pH of Corn Gluten Meal Slurry^a

Hybrid	Drying Treatment				
	Oven			Freeze	Spray
	50°C	100°C	150°C		
Wilson D110	4.08a	4.15b	4.19c	4.31d	4.37d
Pioneer 3394	4.15a	4.20b	4.20b	4.29c	4.35c

^a Means of three millings. Data in the same row with different letters were significantly different at $P < 0.05$.

Nonenzymatic browning not only decreases the availability of proteins, especially in proteins other than zein, but also decreases ammonia released from deamidation, thus decreasing the pH of the CGM slurry. These factors working together could reduce the pH values of oven-dried CGM slurries to values lower than those of the freeze- and spray-dried slurries, as well as the high-temperature-dried CGM.

CONCLUSIONS

CGM samples (two hybrids \times five drying treatments) were used to study the factors influencing zein extraction. Zein extraction yield, protein recovery, and protein purity were higher in the Wilson D110 CGM, which had a high protein content than Pioneer 3394. The yield, protein recovery, and protein purity increased as the oven-drying temperature decreased. Protein aggregation by formation of disulfide bonds at high temperature was likely.

The explanation for the influence of different drying methods on zein extraction is more complex. The freeze- and spray-dried CGM produced lower zein yields, protein recoveries, and protein purities than those obtained for the oven-dried CGM. In addition to the difference in the CGM collection and drying process, protein change during drying was a major factor influencing the yield and protein recovery.

Proteins from CGM treated with low heat (low temperature or short time) were less deamidated during drying. Fewer charged groups on the protein lead to relatively high pH values in the slurries. Aggregation of proteins through noncovalent bonds decreases extractability of α -zeins, and could also be a major influence on zein extraction yield and purity.

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