

## Factors Affecting Yield and Composition of Zein Extracted from Commercial Corn Gluten Meal<sup>1</sup>

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### ABSTRACT

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Twelve corn gluten meal samples obtained from six wet-milling plants were processed into zein. Zein was extracted using 88% aqueous isopropyl alcohol at pH 12.5, followed by chilling. Protein recovery ranged from 21.3 to 32.0%, and protein purity ranged from 82.1 to 87.6%. Protein recovery increased as the protein purity increased ( $r = 0.76$ ) ( $P <$

0.01). One of the major factors influencing extraction yield was protein composition; especially  $\alpha$ -zein content, which ranged from 53.4 to 64% of the total protein in the corn gluten meal samples. The intensity of red color of the corn gluten meal was negatively correlated with protein recovery and zein purity ( $r = -0.66$  and  $-0.72$ , respectively) ( $P < 0.02$ ).

Zein, the predominant storage protein found in maize, was initially designated as a prolamin by Osborne based on its solubility in aqueous alcohol solution and its high content of proline, glutamine, and asparagine (Osborne and Mendel 1914). Zein is a heterogeneous protein separable into four classes based on solubility, amino acid composition, electrophoretic, and immunological properties (Shewry and Tatham 1990, Wilson 1991). The major group of proteins in zein,  $\alpha$ -zeins (MW 19,000 and 22,000 Da), account for  $\approx 71$ –85% of the total zein fraction.  $\beta$ -Zeins (MW 14,000 Da) are 1–5% of the total zein fraction, and  $\gamma$ -zeins (MW 28,000 and 16,000 Da) are 10–20% (Wilson 1991).  $\delta$ -Zeins (MW 10,000 Da) comprise the remaining 1–5%.

Corn gluten meal (CGM) is a valuable coproduct of the wet-milling process and is used primarily as poultry feed because of its high xanthophyll and protein and low fiber contents. CGM contains 60–70% protein, and zein comprises  $\approx 60\%$  (db) of the total protein. Zein isolation from CGM has been studied since the 1930s. Because zein can be dissolved in dilute alkali, Wahl (1934) extracted zein from CGM using a 0.25% NaOH solution. Nykvist (1934) reported that zein was extracted from CGM using a 75–95% ethanol solution at 40–60°C, then precipitated from the alcohol solution by adding salt or acetone. Swallen and his coworkers studied every aspect of the zein extraction process (Swallen and Haute 1938; Swallen 1938, 1941; Horesi et al 1941). This research resulted in a patent and the first commercial process for zein extraction from CGM (Swallen and Haute 1942). CGM was extracted with hot (60°C) aqueous 85% (v/v) isopropyl alcohol, then hexane was added to the clarified extract by removing the oil and pigments. After the hexane layer was removed, zeins were precipitated by adjusting the polarity with cold water, and then spray-dried. The reported yield was 50% based on the weight of the protein content in the CGM, unfortunately, the process used two solvents and was relatively expensive.

Morris and his coworkers (1956 and 1959) developed a process with an extraction solvent containing a low level of organic sol-

vent and  $\text{Ca}(\text{OH})_2$  to successfully extract “whole” zein (including  $\alpha$ -,  $\beta$ -, and  $\gamma$ -zeins) from CGM. The process consisted of an aqueous system containing 28–33% (w/w) isopropyl alcohol and at least 6% lime. The extraction was more effective when the system was heated to a temperature ranging from 70°C to the boiling point of isopropyl alcohol. The amount of total N extracted was  $\approx 75\%$ . After 10 additional purification steps with fresh alcohol solvent, the zein contained only 2% nonprotein impurities. Because the extract did not have to be treated with hexane, the process eliminated the cost of hexane in the defatting step that involved separation and recovery of mixed solvents.

The method most commonly used to extract zein commercially is reportedly a patented process based on Swallen’s research (Carter and Reck 1970). A single extraction is made with 88% (w/w) isopropyl alcohol containing 0.25% NaOH at 55–65°C. The extract is chilled at  $-10$  to  $-20^\circ\text{C}$  to precipitate zeins from the solvent. An increasing zein purity results from repetition of the process using 88% isopropyl alcohol followed by the cooling and decanting cycles. The process does not require separation of zein from the solvent by distillation or an additional extraction with a second solvent (e.g., acetone). Another advantage is that an additional solvent is not required for oil and pigment removal. The operating costs, due to the complex solvent mixture recovery system required, can be decreased, and the operation is reported to be more safe. However, the low yield ( $\approx 20$ –24% of initial CGM weight) and variable quality (e.g., protein content, color intensity, and gelling properties in alcohol solution) of extracted zeins are still problems (Dimitroglou and Breene 1994).

Zein is an excellent candidate for food and nonfood uses because of its unique properties, such as hydrophobicity and film and fiber formation. Methods to improve production yield are extremely important to increase zein utilization. If the factors that influence the yield of zein were identified, yields might be significantly increased. The objectives of this research project were to: 1) extract zein from commercial CGM and compare the yields and purities of zein recovered; and 2) identify the factors that influence zein recovery and quality.

### MATERIALS AND METHODS

#### Corn Gluten Meal Samples

Twelve CGM samples were obtained from six different wet-milling plants in Iowa and Illinois.

#### Extraction of Zein

CGMs were extracted in the lab based on the procedure of Carter and Reck (1970). CGM (30 g) was continuously stirred in a 400-mL beaker with 120 g of extracting solvent containing 88%

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(w/w) isopropyl alcohol and 12% (w/w) pH 12.5 aqueous NaOH at 60°C for 1 hr (Fig. 1). The mixture was centrifuged for 15 min at 8,000 × g in a J2-21 centrifuge (Beckman, Palo Alto, CA); the supernatant was decanted and filtered to remove the alcohol insoluble residue. About 50 mL of the extraction solvent was used to wash the residue. The filtered solution was chilled to -18°C overnight using a refrigerated bath. The zeins formed a taffy-like bottom layer precipitate and the top clear solution was decanted. For purifying zeins, 120 g of 88% aqueous isopropyl alcohol was added to redissolve the zeins at room temperature, followed again by chilling and cool centrifuging. The precipitated zein was dried in a vacuum oven at ≈50°C and 0.6–0.8 bar pressure. The extraction process was replicated five times for each CGM sample.

Zein yield was calculated as the percentage of the extracted zein weight from the total CGM weight (db). Protein recovery was calculated as the percentage of the protein in the extracted zein from the protein of the CGM. Protein purity was defined as the protein content in the extracted zein.

### Proximate Analysis

Moisture contents of the CGM samples were determined by method E203-75 (ASTM 1975) using an automatic titrator (model 392, Fisher Scientific, Pittsburgh, PA). The crude fat content was measured using the Goldfish apparatus (Laboratory Construction Co., Kansas City, MO) according to method 30-20 (AACC 1995). The nitrogen contents in CGM and zein samples were determined by Kjeldahl (Tecator, Sweden) analysis, method A-18 (CRA 1986). The protein content was estimated by using the nitrogen conversion factor of 6.25.

### Color Measurement

The HunterLab Labscan (Hunter, Fairfax, VA) was used to measure the color of the CGM samples. The *L* value indicates the lightness, 0–100 representing dark to light. The *a* value gives the degree of the red-green color, with a higher positive *a* value indicating more red. The *b* value indicates the degree of the yellow-blue color, with a higher positive *b* value indicating more yellow.

### Protein Fraction Isolation and HPLC Analysis

The CGM samples (0.40 g) were extracted in 50-mL centrifuge tubes with 10 mL of 0.5M NaCl solution. The mixture was shaken at 130 rpm for 20 min at room temperature and centrifuged at 30,000 × g for 15 min. The supernatant containing water and salt-soluble proteins was collected, and the extraction procedure was repeated one time. The precipitate was then extracted with 10 mL of 55% (v/v) isopropyl alcohol and 5% (v/v) 2-mercaptoethanol plus 0.5% (w/v) sodium acetate solvent (PMA). The mixture was shaken for 2 hr at room temperature and centrifuged at 30,000 × g for 15 min. The supernatant containing total zeins was collected for high-performance liquid chromatography (HPLC) analysis, and the residue was washed twice with 10 mL of PMA. The protein contents of the water- and salt-soluble fraction and PMA insoluble residue were determined by using the Kjeldahl method.

The reverse-phase (RP) HPLC system consisted of a pump system (model 110A, Beckman, Fullerton, CA), a 214-nm UV detector (ISCO, Lincoln, NE), a microprocessor HPLC solvent programmer-system controller (AXXI-CHROM model 710, Cole Scientific Inc., Los Angeles), and an integrator and printer (model R3A, Shimadzu, Kyoto, Japan). A Vydac (Hesperia, CA) C<sub>18</sub> col-

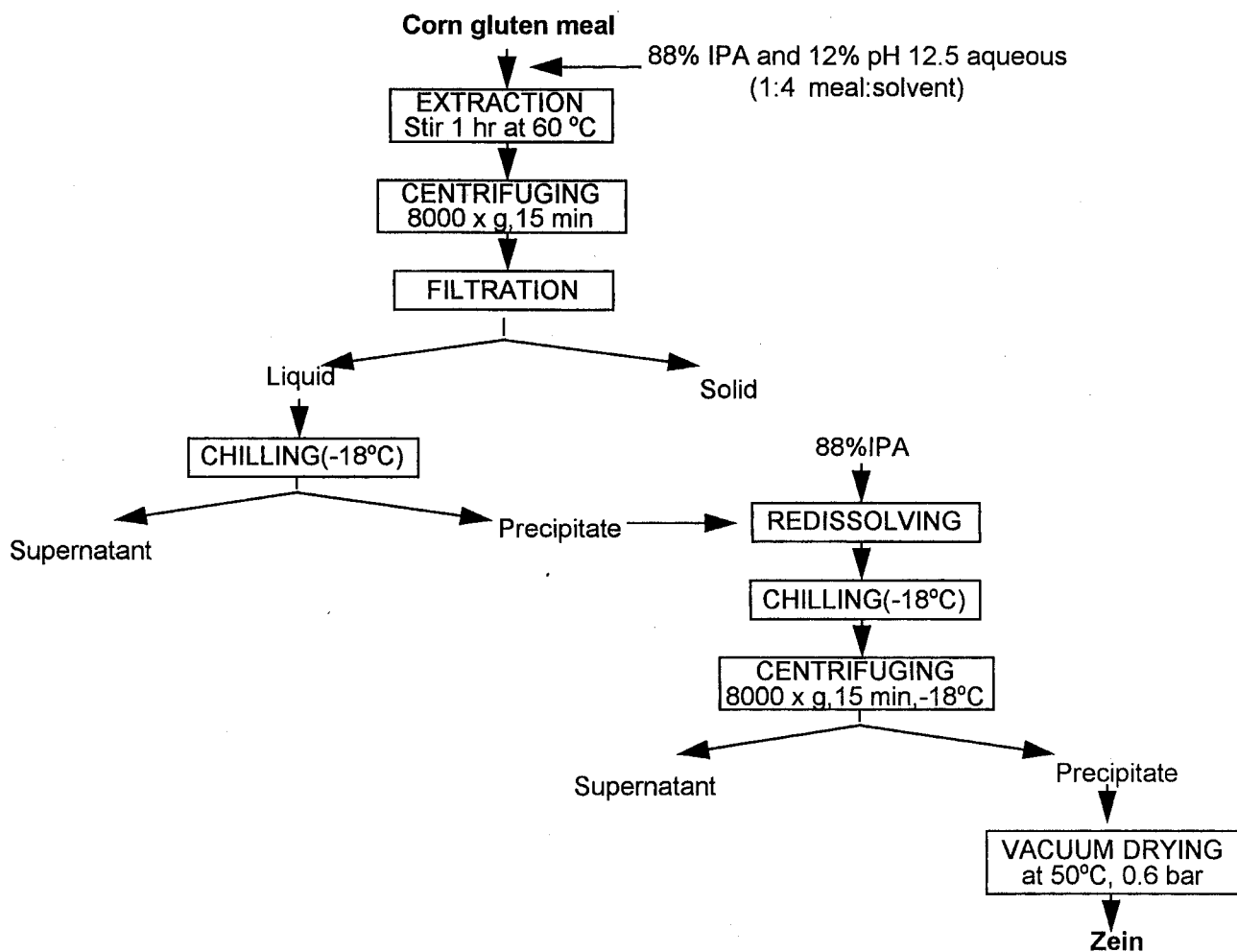


Fig. 1. Laboratory zein extraction diagram.

umn (25 cm × 4.6 mm, 5-μm particle size, 300Å pore size) was used to analyze the α-, β-, and γ-zein contents in the samples (Dombrink-Kurtzman and Bietz 1993). A 20-μL sample (≈25 μg of protein) was injected for analysis. Solvent A [15% (v/v) acetonitrile (ACN) plus 0.1% (v/v) trifluoroacetic acid (TFA)] and solvent B [80% ACN plus 0.1% TFA] were used to make a nonlinear gradient. The starting buffer was 38.4% ACN, increasing at 0.8125%/min for 10 min, 0.093%/min for 7 min, 0.609%/min for 8 min, 0.186%/min for 35 min, and 1.95%/min for 3 min, ending at 64.4% ACN. The column was eluted at 56°C and 1.0 mL/min solvent flow rate.

### Statistical Analysis

Analysis of variance was used to determine significant effects using the 5% significant level for least significant differences (LSD). Correlation coefficients were calculated by using the SAS correlation procedure (SAS 1987).

## RESULTS AND DISCUSSION

### Proximate Composition and Color of the CGM

The CGM samples differed significantly in proximate composition, most notably in protein content and color (Table I). Protein contents of the CGM samples ranged from 61.5 to 74.0% (db). In regard to color, the samples looked obviously different. Lightness of the CGM samples was highly correlated with yellow color ( $r = 0.95$  for  $L$  and  $b$  values at  $P < 0.0001$ ), and negatively correlated with the red color ( $r = -0.54$  for  $L$  and  $b$  values at  $P < 0.1$ ). The differences in the proximate composition and color may be caused by the corn hybrids milled, corn treatment and storage, the wet-milling process, and CGM drying conditions.

TABLE I  
Proximate Compositions and Colors of Commercial Corn Gluten Meal (CGM) Samples<sup>a</sup>

CGM	Moisture (%)	Fat (% db)	Protein (% db)	Color		
				<i>L</i>	<i>a</i>	<i>b</i>
A	7.19h	1.08gh	73.0b	55.86b	4.38g	23.37ef
B	8.78e	1.57e	68.7f	50.37f	6.48e	23.14f
C	5.94j	1.14g	66.8g	45.63g	7.37bc	20.90g
D	6.67i	1.68de	66.3h	52.22e	7.46bc	23.91e
E	10.10b	1.89c	66.2h	54.35d	7.04d	24.88d
F	9.54c	1.06gh	71.6c	55.42cd	7.62b	25.50cd
G	11.72a	2.35a	74.0a	56.95b	7.66b	26.29b
H	9.29d	1.71d	69.8d	50.44f	8.60a	23.44ef
I	7.51g	1.74d	65.5i	43.56h	8.52a	19.64h
J	8.05f	1.40f	69.1e	52.11e	7.69b	23.62ef
K	8.76e	2.08b	61.5j	64.11a	5.72f	27.75a
L	8.07f	0.91i	69.1e	56.84bc	7.12cd	25.90bc

<sup>a</sup> Means followed by different letters within the same column are significantly different ( $P < 0.05$ ).

TABLE II  
Yields, Protein Purities and Protein Recoveries of Extracted Zein from Corn Gluten Meal (CGM) Samples<sup>a</sup>

CGM	Yield, %	Protein Purity, %	Protein Recovery, %
A	26.6a	87.6a	32.0a
B	24.1b	86.3bc	30.3ab
C	21.7cd	85.8cd	27.9bc
D	22.5bc	84.8def	28.9b
E	22.6bc	87.5ab	29.8ab
F	22.3bc	84.1efg	26.2cd
G	22.5bc	84.3efg	25.6cd
H	18.8ef	82.1h	22.1ef
I	17.2f	84.0fg	21.9ef
J	20.2de	83.1gh	24.3de
K	18.6ef	84.8def	25.6cd
L	17.3f	85.4cde	21.3f

<sup>a</sup> Means followed by different letters within the same column are significantly different ( $P < 0.05$ ).

### Zein Extraction Yield and Purity

Zein yield ranged from 17.2 to 26.6%, while protein purity ranged from 82.1 to 87.6% (Table II). There were significant differences in zein yield and purity among the samples ( $P < 0.001$ ). Over 70% of the error in zein extraction yield and purity was contributed by the CGM samples, and the rest of the error was contributed from replication.

There was a linear trend between the protein recovery and zein purity (Fig. 2). The protein recovery increased significantly as protein purity increased ( $r = 0.76$ ,  $P < 0.01$ ). The relationship of zein yield and protein purity had a linear trend similar to that of protein recovery. The zein yield positively correlated with the protein purity ( $r = 0.61$ ,  $P < 0.05$ ). Thus, when the yield of extracted zein is high, the extracted zein has a high protein content.

### Influence of Red Color on Zein Recovery and Purity

The results show a relationship between the level of red color in the CGM and the yield and purity of zein extracted. Figure 3 shows that the protein recovery decreased as the  $a$ -value of CGM increased. The correlation coefficient between protein recovery and  $a$ -value of the 12 samples was  $-0.66$  (Fig. 3) with a significance level of  $P < 0.02$ . The zein yield also decreased as the  $a$ -value of CGM increased ( $r = -0.57$ ,  $P < 0.1$ ). Figure 4 shows that protein purity was also significantly negatively correlated with red color, which indicates that less protein or more impurities were extracted from CGM high in red color ( $r = -0.72$ ,  $P < 0.01$ ). This result could be explained by high temperature drying of the CGM. Because the red color is likely intensified during drying of the CGM, a larger  $a$ -value may be an indication of chemical browning during more "severe" drying.

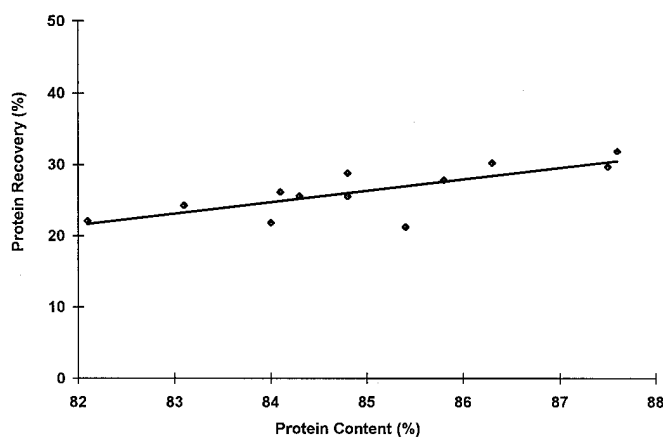


Fig. 2. Relationship between protein recovery and purity of extracted zein.

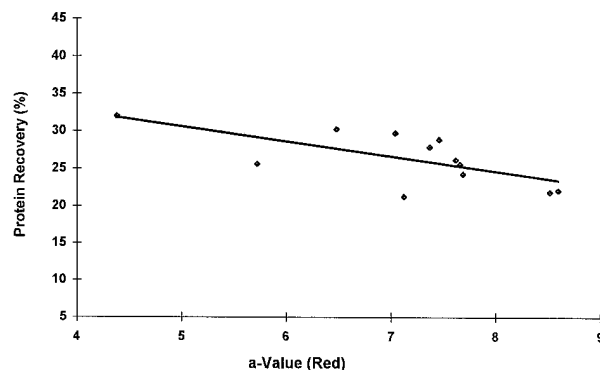


Fig. 3. Relationship between protein recovery and the red color of the corn gluten meal samples.

Because the wet-milling plants where the CGM samples were collected used different hybrids of corn (with varying levels of color) and have different steeping, milling, and drying (May 1991) procedures, it is difficult to establish a relationship between CGM red color and zein yield and purity. Furthermore, data with stronger statistical evidence will also be required to make any firm conclusions. However, the trends in the data clearly show that of the CGM samples tested, samples higher red color gave lower zein yields and purity, and the intensity of the red color may be an indirect parameter to measure the loss of zein extraction capability.

The moisture and fat contents in the CGM ranged from 5.94 to 11.72% and 0.91–2.35% (Table I), respectively, and did not influence the zein extraction yield or purity.

### Influence of Zein Content of CGM on Zein Extraction Yield

The protein fraction analysis measured the level of water- and salt-soluble, alcohol-soluble (plus reducing agent), and alcohol-insoluble proteins present in CGM. The differences in the levels of these protein fractions between CGM samples were significant (Table III). Alcohol-soluble proteins, zeins, ranged from 40 to 50% (db) of the total weight of the CGM and were 60–71% of the protein content in CGM. The water- and salt-soluble proteins were only 2–6% (db) of the weight of CGM. The alcohol-soluble and salt-soluble protein content in CGM were similar to results reported by Neumann et al (1984).

Small amounts of  $\beta$ -zein (0.7–2.9%) and  $\gamma$ -zein (4.7–10.6%) were present in the CGM, but much higher contents of  $\alpha$ -zein (86.1–93.8%) were presented in the CGM samples (Table IV). The ratio of  $\alpha$ -zein to the protein content of CGM, calculated by

**TABLE III**  
Protein Composition (%) of Corn Gluten Meal (CGM) Samples<sup>a</sup>

CGM	Albumins/ Globulins	Zeins	Alcohol-Insoluble Proteins
A	2.8g	49.8a	20.4e
B	3.4f	44.8d	20.5de
C	4.5c	41.8e	20.5de
D	4.2cd	39.8g	22.3bc
E	3.7ef	47.1b	15.4g
F	2.1hi	46.8b	22.7b
G	6.1a	47.0b	20.9d
H	2.5gh	45.4c	21.9c
I	1.9i	41.7e	21.9c
J	2.0i	41.5ef	25.6a
K	5.0b	41.1f	15.4g
L	4.0de	45.4c	19.7f

<sup>a</sup> Means followed by different letters within the same column are significantly different ( $P < 0.05$ ).

**TABLE IV**  
Zein Content (%) of Corn Gluten Meal (CGM) Samples<sup>a</sup>

CGM	Total Zeins			$\alpha$ -Zeins/CGM Protein Ratio <sup>b</sup>
	$\alpha$ -Zeins	$\beta$ -Zeins	$\gamma$ -Zeins	
A	93.8a	0.74e	4.67e	64.0
B	91.0bc	1.66bc	5.56de	59.3
C	89.4cd	1.03de	8.07b	55.9
D	89.0cd	2.18b	6.86bcd	53.4
E	88.5de	1.97c	7.20bc	63.0
F	87.6de	2.12b	7.71b	57.3
G	89.1cd	2.94a	5.49de	56.6
H	89.6bcd	2.07b	6.58bcd	58.3
I	86.1e	1.39cd	10.58a	54.8
J	92.0ab	1.35cd	5.44de	55.3
K	87.7de	1.81bc	8.04b	58.6
L	92.0ab	1.03de	5.76cde	60.4

<sup>a</sup> Means followed by different letters within the same column are significantly different ( $P < 0.05$ ).

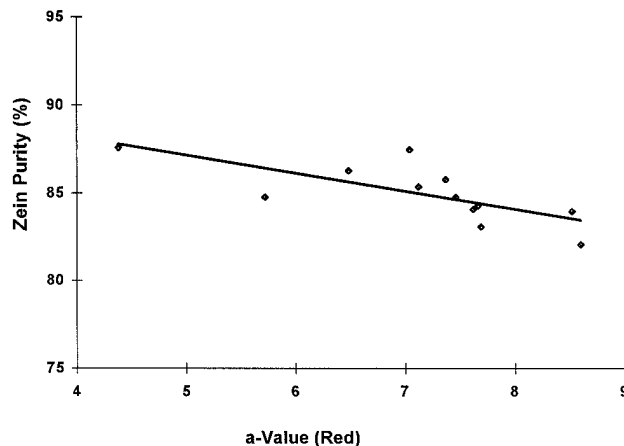
<sup>b</sup> Ratio of  $\alpha$ -zein to the protein content of CGM, calculated by dividing the amount of  $\alpha$ -zeins by the protein content of the CGM.

dividing the amount of  $\alpha$ -zeins by the protein content of the CGM, ranged from 53.4 to 64.0% among CGM samples.

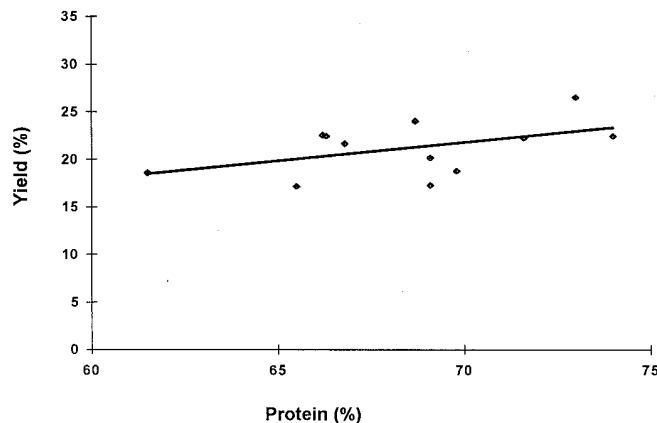
The differences in the total extractable zein and  $\alpha$ -zein contents among samples could be attributed to the corn hybrids that were milled (Wilson 1991, Dombrink-Kurtzman and Bietz 1993). The differences in the way that corn gluten was dried may also account for these differences because of protein denaturation.

The protein content of CGM in our investigation was significantly correlated with total zein content ( $r = 0.72$ ) and the total  $\alpha$ -zein content ( $r = 0.75$ ,  $P < 0.01$ ). Zein yield was positively correlated with both zein and  $\alpha$ -zein content. Both plots of zein yield and to protein and  $\alpha$ -zein content of the CGMs showed an increased linear trend in the data (Figs. 5 and 6). The relationship of zein extraction yields to total  $\alpha$ -zein contents was  $r = 0.55$  ( $P < 0.1$ ), compared to  $r = 0.48$  ( $P < 0.12$ ) between yields and the total protein level. Although the data shows that the correlation between zein yield and  $\alpha$ -zein contents was more significant than yield and total protein, a relatively small data set was used in this study (12 samples); thus, more data will have to be collected to confirm this result.

The solubility of zeins in aqueous alcohol differ according to class.  $\alpha$ -Zeins and  $\delta$ -zeins can be easily extracted with 90% isopropyl alcohol.  $\beta$ -Zeins and  $\gamma$ -zeins cannot be extracted with 90% isopropyl alcohol solution, but they can be extracted along with  $\alpha$ -zeins using 60% isopropyl alcohol (Esen 1986). Furthermore,  $\beta$ -zeins and  $\gamma$ -zeins both occur as large homo or hetero oligomers and multimers linked through intermolecular disulfide bonds, and typically require a reducing agent (e.g., 2-mercaptoethanol) to be



**Fig. 4.** Relationship between the zein extraction purity and the red color of the corn gluten meal samples.



**Fig. 5.** Relationship between the zein extraction yield and the protein content of the corn gluten meal samples.

extracted. When using 88% isopropyl alcohol without a reducing reagent, as with the extraction solvent in our procedure, the extraction product should contain almost exclusively  $\alpha$ -zeins and possibly tiny amounts of  $\delta$ -zeins.

The results of the aforementioned data show that zein extraction yield is significantly correlated to both protein and  $\alpha$ -zein content. Therefore, a measurement of both total protein and  $\alpha$ -zein content in the CGM might be considered a better predictor of zein extraction yields and purity.

### The Relationships of Red Color and $\alpha$ -Zein Content with Zein Yield and Purity

Three-dimensional scatter graphs show the relationships of  $\alpha$ -zein content and red color intensity in the CGM with zein extraction yield and protein purity. Zein yield was the highest at the highest  $\alpha$ -zein content and the lowest red color intensity, and the lowest at the lowest  $\alpha$ -zein content and the highest red color intensity in the CGM (Fig. 7). Both red color intensity and  $\alpha$ -zein content in CGM function together and affect extraction yield.

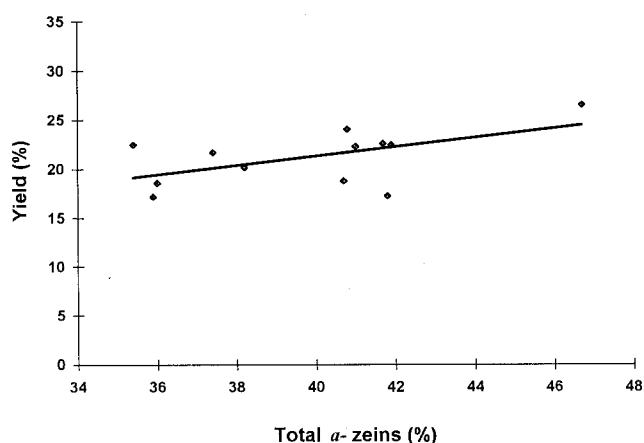


Fig. 6. Relationship between the zein extraction yield and the total  $\alpha$ -zein content in the corn gluten meal samples.

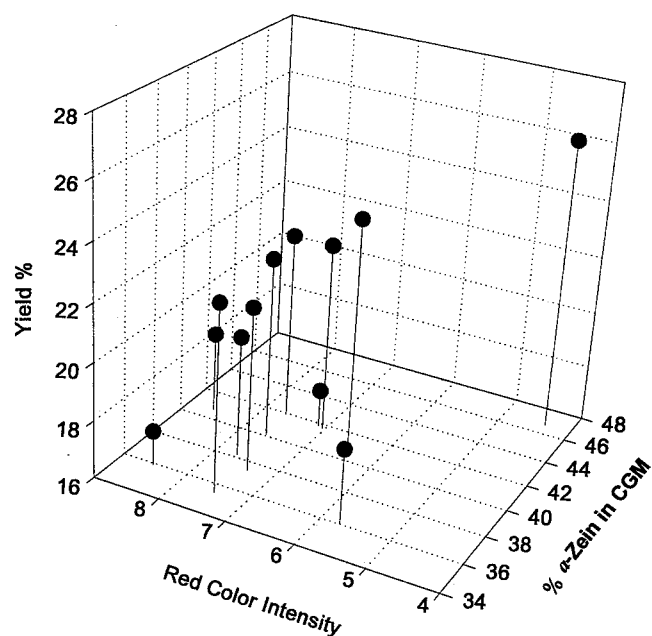


Fig. 7. Relationship between extraction yield and the total  $\alpha$ -zein content and red color intensity of the corn gluten meal samples.

When a low yield was obtained from a relatively high red color CGM with high  $\alpha$ -zein content, the red color may be caused by overheating during drying. When a relative high yield was obtained from high red color CGM with high  $\alpha$ -zein content, the red color may be caused by the corn hybrids milled. Protein recovery had similar associations with  $\alpha$ -zein content and red color intensity in CGM as the yield. Lower red color intensity in CGM gave the higher protein purity, and the protein purity also increased as the  $\alpha$ -zein content increased (Fig. 8).

### Extraction Efficiency from Commercial CGM

Comparing the protein recovery value (Table II) with the ratio of  $\alpha$ -zeins to the protein content of the CGM (Table IV), this extraction method only obtained  $\approx 35$ –50% of the extractable  $\alpha$ -zeins in the CGM. The low extraction efficiency may be due to disulfide bonds present among zeins and other proteins because no reducing reagent was used during extraction. Some zeins form oligomers or polymers by cross-linking disulfide bonds which impede the extraction with alcohol (Neumann 1984). The lower extraction efficiency may also be due to hydrophobic interactions between zeins and other proteins, or other compounds such as lipids, decreasing the solubility of zein in alcohol solution.

This information compares favorably with information found in the literature and strongly suggests that although the variation in zein yield and purity can be improved by controlling factors such as CGM protein content, the increase in yield will only be gained by the development of a new method of extracting zein.

## CONCLUSIONS

Zein was extracted from 12 CGM samples with a high temperature ( $60^{\circ}\text{C}$ ) solvent extraction process followed by chill separation. Zein yield and protein recovery were positively correlated with zein purity. As the total and  $\alpha$ -zein content, extracted using PMA solvent and measured with HPLC, increased in the CGM samples, zein yield and protein recovery using the aforementioned extraction process, also increased. High red color of the CGM had a negative effect on the zein extraction yield, protein recovery and zein purity. Both  $\alpha$ -zein content and red color intensity function together to affect zein extraction yield and purity.

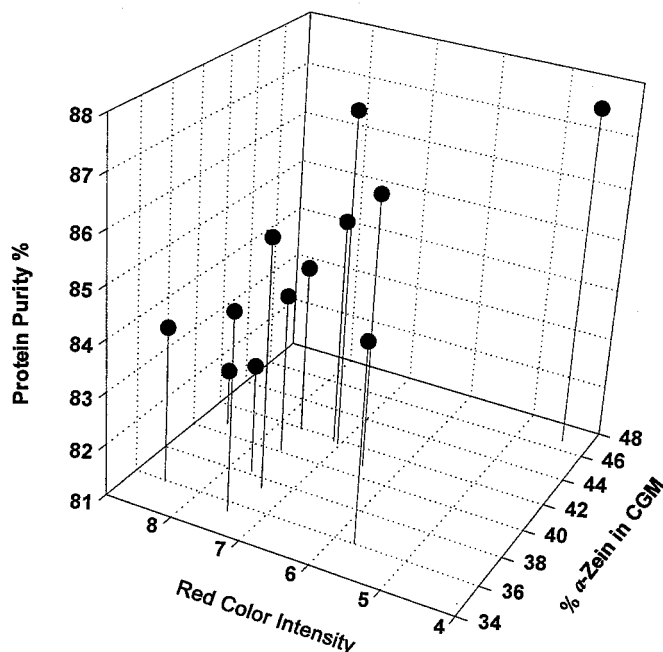


Fig. 8. Relationship between protein purity of extracted zein and the total  $\alpha$ -zein content and red color intensity of the corn gluten meal samples.

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