

# Solvent Extraction of Zein from Dry-Milled Corn

Rishi Shukla,<sup>1</sup> Munir Cheryan,<sup>1,2</sup> and Richard E. DeVor<sup>3</sup>

## ABSTRACT

Cereal Chem. 77(6):724–730

Batch extraction of zein from dry-milled whole corn with ethanol was optimum with 70% ethanol in water, an extraction time of 30–40 min, and temperature of 50°C. High yields (60% of the zein in corn) and high zein contents in the extracted solids (50%) were obtained at a solvent-to-solids ratio of 8 mL of 70% ethanol/g of corn. However, zein concentration in the extract was higher at lower ratios. Multiple extraction of the same corn with fresh ethanol resulted in a yield of 85% after four extrac-

tions, whereas multiple extractions of fresh corn with the same ethanol resulted in high (15 g/L) zein concentration in the extract. Optimum conditions for batch extraction of zein were 45°C, with 68% ethanol at a solvent-to-solids ratio of 7.8 mL/g for an extraction time of 55 min. Column extractions were also best at 50°C and 70% ethanol; a solvent ratio of 1 mL/g resulted in high zein concentrations in the extract (17 g/L) but yields were low (20%).

Zein and glutelin constitute the bulk of the storage protein in corn (Reiners et al 1973). Zein (or zēine) was first identified in 1821 by infusing water in *zea* or Indian corn. It has been the subject of numerous studies since the 1890s and is principally characterized by its solubility in aqueous alcohol solutions. It is soluble in water, but only under high concentrations of urea or alkali ( $\geq$ pH 11), in the presence of anionic detergents (Turner et al 1965), or after enzymatic modification (Mannheim and Cheryan 1993).

Although it has GRAS status (Anonymous 1985), zein is not rated for human consumption because it results in a negative nitrogen balance when used as the sole dietary protein. The zein that is available commercially today is produced from corn gluten meal and is different from the zein present in whole corn (Boundy et al 1967). Zein has a wide range of applications (Lower 1999; Shukla and Cheryan, *unpublished*): fibers, adhesives, ceramics, coatings (it is comparable to shellac), inks, cosmetics, membranes for seawater desalination, chewing gum, pharmaceutical tablets, and biodegradable plastics (Lai et al 1997). However, large-scale use of zein has been hindered in part by its high cost, which can range from \$10 to \$44/kg, depending on purity and method of manufacture.

Most methods of zein manufacture have focused on solvent extraction of corn gluten meal using low-carbon alcohols (Mossé 1961). Quality of zein varies due to batch-to-batch differences in corn gluten meal. With degermed and dehulled corn, Russell and Tsao (1982) extracted >80% of the zein using a batch extractor. Lawhon (1986) and Kampen (1995) proposed sonication and wet attrition milling, respectively, to extract protein (zein and nonzein fractions) from whole corn. Chen and Hoff (1987) patented a combined oil and zein extraction process from dried ground corn using 50–100% ethanol. Protein recovery and ethanol recycle was accomplished by centrifugation. Hojilla-Evangelista et al (1992) extracted >70% of the total protein from deoiled corn with 45% ethanol and 55% 0.1*N* sodium hydroxide. This was followed by evaporation to recover protein. Dickey et al (1998, 1999) extracted zein from whole ground corn with 70% ethanol followed by water displacement to recover zein and distillation of ethanol for recycle. They were successful in producing relatively pure zein (>80%) at the relatively low cost of  $\approx$ \$3.09/kg. Zein recovery was  $\approx$ 48% of that in the corn.

Several factors affect the yield and purity of zein extracted from corn or its coproducts, such as time of extraction, temperature of

extraction, ethanol concentration, and the relative amounts of solvent and corn solids. No comprehensive study has been made on the effect of these variables, especially the interactions among these variables, on extraction of zein from corn. Few studies have reported the effect of solvent-to-solids ratio, which is an important parameter because the major determinants of the final cost of zein are solvent removal, recovery, and yield of zein.

This study was undertaken to understand and optimize the effect of these variables on the yield of zein, zein concentration in the extract, and its purity. Experiments were performed in three phases. An initial screening study on batch extraction of whole ground corn was conducted with the four variables. A near optimum was determined for each parameter from the study. Because of the many variables and the corresponding interactions, a statistical technique was employed to determine optimum conditions of extraction (Box et al 1978). A second-order experimental design was used to determine the response surface of zein concentration in the extract near the zone of optimum, which provided the basis for a second-order polynomial approximation. Finally, as solvent extraction of corn would most likely be performed in a continuous mode, the optimum conditions obtained from batch extraction studies were used to simulate a continuous extraction.

The ultimate objective of this research is to provide an additional high-value coproduct for dry mill ethanol plants. These plants produce 40–50% of the ethanol used in the United States today. The products of dry milling (distillers grains, ethanol, and carbon dioxide) return about half the value of corn (Keim 1995), depending on the cost of corn. When plant depreciation is included in the cost analysis, most dry mills would not be economically viable without tax waivers and other incentives. However, the revenue earned from higher value coproducts should improve their economic viability and perhaps make subsidies unnecessary. Thus, this research focused on using dry-milled whole corn as the raw material.

## MATERIALS AND METHODS

Whole ground corn (yellow dent #2) was obtained from a dry-mill ethanol plant in the Midwest and used without further sieving. Ethanol (anhydrous, 200 proof) was obtained from McCormick Distillation Co., (Weston, MO). Aqueous solutions of ethanol were prepared on a v/v basis. Deionized water was used for all experiments. Commercial zein samples were obtained from Freeman Industries (Tuckahoe, NY) (F4000) and from Showa Sangyo (Kyoto, Japan).

Particle-size distribution of the milled corn was determined in a ro-tap shaker with U.S. standard sieves. Moisture content of corn was determined gravimetrically by oven drying at 135°C for 2 hr. Total solids in extracts were determined by oven drying at 103°C overnight (Dickey et al 1997). Nitrogen (N) was determined by the Kjeldahl analysis using Approved Method 46-08 (AACC

<sup>1</sup> Agricultural Bioprocess Laboratory, University of Illinois at Urbana-Champaign, 1302 West Pennsylvania Avenue, Urbana 61801.

<sup>2</sup> Corresponding author. Phone: 217-333-9332. Fax: 217-333-9592. E-mail: mcheryan@uiuc.edu

<sup>3</sup> Department of Mechanical and Industrial Engineering, University of Illinois, Urbana.

2000). Protein is expressed as  $N \times 6.25$  (AOAC 1984). These analyses were performed in triplicate and the mean value is reported.

Zein is defined as the alcohol-soluble protein in corn. Zein content of whole corn was determined by doing two extractions at a solvent-to-solids ratio of 12:1 with 70% ethanol at 50°C for 18 hr each.

Yield of zein in the extract is defined as:

$$\text{Yield (\%)} = \frac{[\text{zein concentration of extract (g/L)} \times \text{volume of extract (L)}]}{\text{zein content of corn (g)}} \times 100 \quad (1)$$

Zein content of extracts (db) is defined as:

$$\text{Zein content (\%)} = \frac{[\text{zein concentration of extract (g/L)} / \text{total solids of extract (g/L)}] \times 100 \quad (2)$$

### Batch Extraction

Stirred batch extractions were performed by adding 50 g of dry-milled corn to the appropriate volume of preheated aqueous ethanol. Extractions were done in an Erlenmeyer flask with a magnetic stirrer (300 rpm) placed in a water bath and fitted with a cold water condenser to minimize ethanol vapor loss. After completing the extraction, corn solids were separated from corn-ethanol slurry by filtering under vacuum through a Whatman #1 filter paper rated at 11- $\mu\text{m}$  particle retention size. The volume of extract obtained after filtration was recorded. All fractions were stored at 4°C until analyzed for total solids and protein.

### Column Extraction

Column extraction was performed by packing 100 g of dry-milled corn in a 2.54- $\times$  30-cm jacketed glass column and pre-heating the corn to the temperature under study. Ethanol solvent was then pumped through the column using a peristaltic pump with residence times of 25–30 min. The parameters studied were temperature, ethanol concentration, and solvent-to-solids ratio. To study the effect of solvent-to-solids ratio, four separate 100-mL batches of ethanol solvent were pumped through the column. Each of the extracts eluted was collected separately and volume, total solids, and protein were measured.

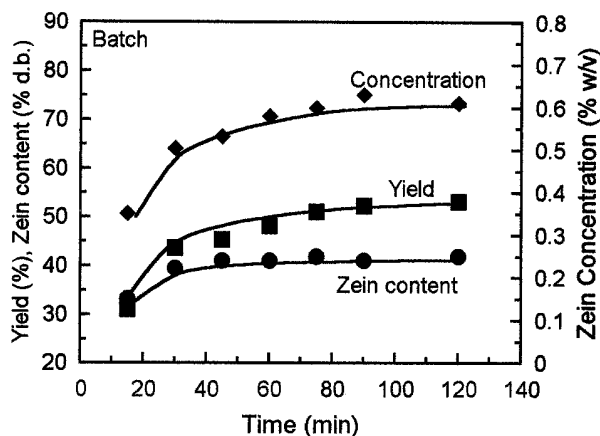


Fig. 1. Effect of time on batch extraction of zein. Solvent-to-solids ratio 4:1, 70% ethanol, 50°C.

TABLE I  
Levels of Variables for Central Composite Design

Symbol	Variable	-2	-1	0	+1	+2
$x_1$	Temperature (°C)	20	30	40	50	60
$x_2$	Ethanol (% v/v)	50	60	70	80	90
$x_3$	Ratio (mL/g)	2	4	6	8	10
$x_4$	Time (min)	15	30	45	60	75

Multiple extractions were done to simulate continuous extraction. A series of batch extractions were done in two modes: 1) extracting the same batch of corn with multiple volumes of fresh ethanol; and 2) extracting fresh batches of corn with the same volume of ethanol. In both cases, experimental conditions remained constant (50°C, 70% ethanol, 8:1 solvent-to-solids ratio, and 30 min of extraction time). In the latter case, because fresh corn absorbed  $\approx 0.76$  mL of ethanol solvent/g of corn, subsequent extractions used correspondingly less fresh corn to maintain the solvent-to-solids ratio at 8:1.

### Factorial Design

Based on the results obtained from screening studies, a 2<sup>4</sup> full-factorial design was constructed to model the experiment. A first-order model obtained from the design displayed lack-of-fit. Therefore, subsequent experiments were performed at additional points using a central composite design (Box et al 1978) to model response using higher order models. The treatment levels for the central composite design are shown in Table I. Six replicates at the center were used to estimate variances. Analysis of variance (ANOVA), response surface plots, and estimates for main-factor and two-factor interactions were determined using Statgraphics Plus 6.1 (Manugistics Inc., Rockville, MD). The location of the optimum from the second-order model (Shukla 2000) was obtained using Matlab 5.3 (The Mathworks Inc., Natick, MA).

### Electrophoresis

SDS-PAGE was conducted using a Mini Protean II gel electrophoresis cell (BioRad Laboratories, Hercules CA) using 10–20% Tris-glycine gels purchased from BioRad. Tris/glycine/SDS 2 $\times$  running buffer and Laemmli premixed sample buffer (Biorad) were used. Samples were run at 70V for 4 hr after which the gels were stained in Coomassie Brilliant Blue R-250 solutions for 2 hr. The gels were then destained in 20% acetic acid and 10% ethanol solutions overnight.

## RESULTS AND DISCUSSION

Over 70% of the particles in the milled corn received from the dry grind ethanol company were 200–800  $\mu\text{m}$ . A major goal of this research was to incorporate this process into existing plants. Therefore, no further size reduction was done and the corn was used as is. Protein content of the corn was 7.3–7.8% (as-is basis) between batches and moisture content was 14.5–16.5%. Zein content averaged 50.6% of the protein in corn.

Screening studies for time, temperature, and ethanol concentration were made at solvent-to-solids ratios of 8:1 and 4:1. Zein

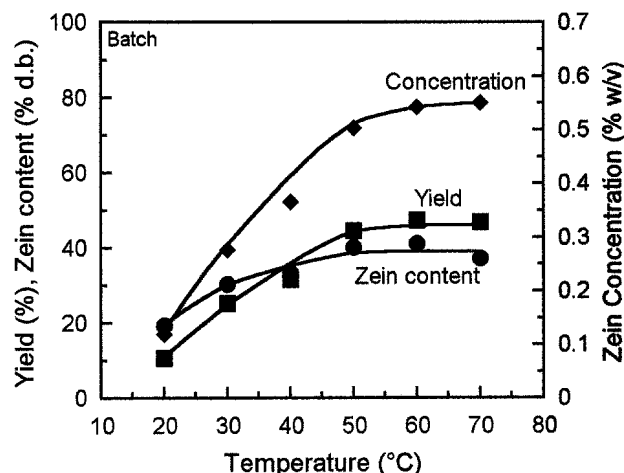


Fig. 2. Effect of temperature on batch extraction of zein. Solvent-to-solids ratio 4:1, 70% ethanol, 30 min.

extraction stabilized after  $\approx 45$  min (Fig. 1). Zein concentration in the solvent actually did not reach a maximum because all the zein in corn is solubilized only with long extraction times (Russell 1980). Results at both 8:1 and 4:1 solvent-to-solids ratios were similar (Shukla 2000). A material balance showed that the corn absorbed, on average, 0.76 mL of solvent/g of corn (average of 42 experiments). Thus, batch experiments could not be conducted at solvent-to-solids ratios  $< 2$  due to practical difficulties in stirring the slurry. This phenomenon was also reported by Russell (1980). In addition, experiments with low concentrations of alcohol ( $< 50\%$ , v/v) in combination with high temperatures ( $> 65^\circ\text{C}$ ) were not possible due to gelation of the starch in corn.

Experiments investigating the effect of temperature (Fig. 2) indicate that zein solubilization was maximum at  $50^\circ\text{C}$ . This is because transfer of zein from corn grits is rate-limited by transport of zein out of the endosperm (Russell and Tsao 1982, Dickey et al 1998) and the diffusion coefficient of a protein increases  $\approx 3\text{--}3.4\%$ / $^\circ\text{C}$  rise in temperature (Cheryan 1998). Temperatures  $> 50^\circ\text{C}$  did not result in higher yields, possibly due to protein denaturation. Mossé (1961) also reported similar behavior with corn endosperm. Hojilla-Evangelista (1990) found that  $55^\circ\text{C}$  was the optimum temperature for extraction, and temperatures  $> 65^\circ\text{C}$  resulted in protein denaturation.

The effect of ethanol concentration on extraction parameters is shown in Fig. 3. A distinct maximum in protein solubilization and yield was observed at 70% ethanol. This was also observed at 8:1 solvent-to-solids ratio (Shukla 2000). Extractions with 40 and 50% ethanol yielded turbid extracts that did not become clear on

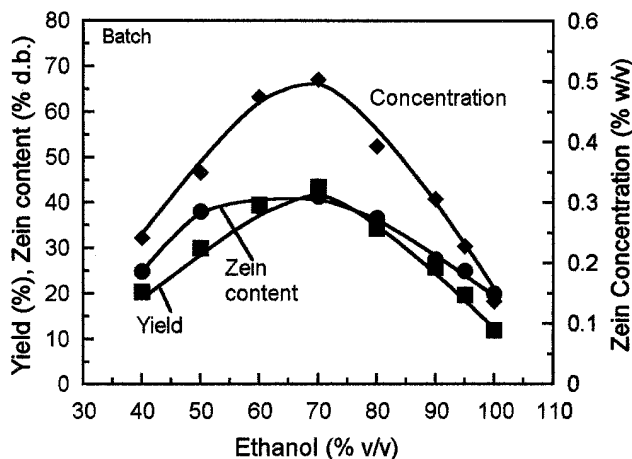


Fig. 3. Effect of ethanol concentration on batch extraction of zein. Solvent-to-solids ratio 4:1, 30 min,  $50^\circ\text{C}$ .

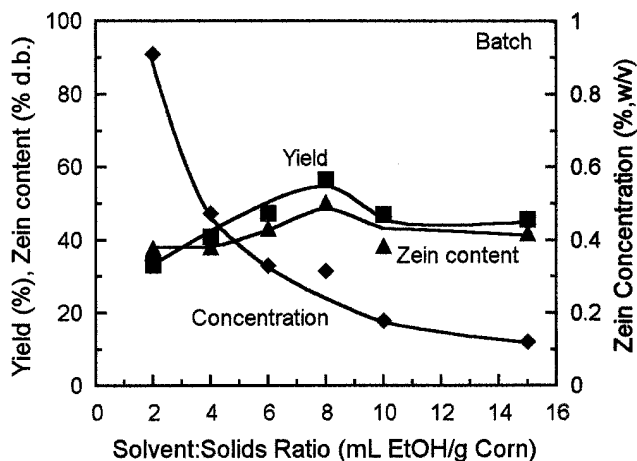


Fig. 4. Effect of solvent-to-solids ratio on batch extraction of zein. 70% ethanol,  $50^\circ\text{C}$ , 30 min.

standing overnight at  $4^\circ\text{C}$ , although no precipitates were observed. Even 40% ethanol extracted a small amount of zein, which suggests that water displacement methods might not completely precipitate zein in extracts. Zein recoveries with 95 and 100% ethanol were quite low ( $< 20\%$ ) and lower than reports by Hojilla-Evangelista (1990), who predicted recoveries of 33.5 and 24.7%, respectively, at  $40^\circ\text{C}$  from defatted corn. This is possibly due to a difference in milling conditions, extraction procedure, or particle size, or perhaps because corn oil hinders protein extraction.

Zein is located in 1- $\mu\text{m}$  zein bodies distributed throughout the cytoplasm of endosperm cells between starch granules of 5–35  $\mu\text{m}$ . Of two prolamines,  $\alpha$ -zein and  $\beta$ -zein (McKinney 1958),  $\alpha$ -zein is soluble in 95% ethanol or 85% isopropanol and represents  $\approx 80\%$  of the prolamine content, whereas  $\beta$ -zein is the fraction that is soluble in 60% ethanol and insoluble in 95% ethanol. The  $\beta$ -zein fraction constitutes  $\approx 20\%$  of the total prolamine (Reiners et al 1973). The  $\beta$ -zein is less stable in solution, due perhaps to a higher content of sulfur-containing amino acids, which results in a tendency to gel easily. The protein bodies from cells in the inner part of the kernels are larger and contain mostly  $\alpha$ -zein, whereas protein in the outer, subaleurone layer contain mostly  $\beta$ - and  $\gamma$ -zeins (Duvick 1961, Lending and Larkins 1989). This sort of arrangement would allow the  $\beta$ -zein in the outer layer to be preferentially exposed to ethanol solutions. Dry grinding of corn alone will not break the matrix. Furthermore, in the absence of a disulfide reducing agent,  $\alpha$ -zein will be prevented from solubilizing, thus leading to lower protein concentrations and yields.

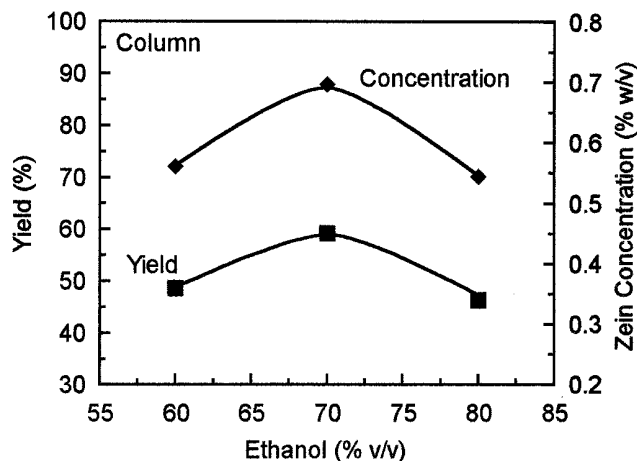


Fig. 5. Effect of ethanol concentration on column extraction of zein. Solvent-to-solids ratio 4:1,  $50^\circ\text{C}$ , 25 min.

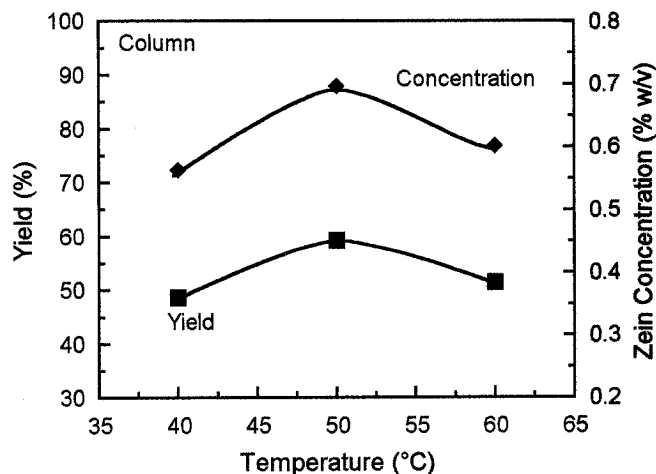


Fig. 6. Effect of temperature on column extraction of zein. Solvent-to-solids ratio 4:1, 70% ethanol, 25 min.

The effect of solvent-to-solids ratio on yield and concentration of zein is shown in Fig. 4. Zein yield and purity were highest at 8:1. Lower ratios result in higher concentrations of protein in the extract but lower yields. The lower protein concentrations at higher ratios can be explained by protein dilution (Chen and Houston 1970, Wu and Sexson 1976). A solvent-to-solids ratio of 4:1 or 6:1 appears to be good compromise between yield and zein concentration in extract. This value is close to that reported by Wu and Sexson (1976), who found a ratio of 6:1 was optimal for alkaline extraction of corn endosperm. Higher costs of evaporating ethanol offset the benefits of slightly higher yield and purity with higher solvent-to-solids ratios.

The 60% yield reported here at 6:1 is lower than reported by Russell and Tsao (1982), who obtained a yield of 80% zein. However, their dry-milled corn was extracted at higher solvent-to-solids ratios (12:1) with longer periods of extraction (18 hr). Kampen (1995) and Lawhon (1986) reported  $\geq 80\%$  of total protein from corn was solubilized. In both these cases, however, the data is based on total protein because zein as well as nonzein proteins from corn were extracted.

### Column Extraction

Results of column extraction of ground corn are shown in Figs 5 and 6. A near optimum at 50°C and 70% ethanol concentration was obtained, similar to batch extraction. However, a packed column extractor allows the use of lower solvent-to-solids ratios (Fig. 7). This results in a higher concentration of zein (1.7%, w/v) at a 1:1 ratio, compared with 0.9% (w/v) at a 2:1 ratio in a batch process. However, high yield and high concentration are mutually exclusive with this method. Increasing the ratio increased the yield but lowered the zein concentration. Chen and Hoff (1987) obtained concentrations of 2.8% (w/v) and a yield of 40% based on total protein present in defatted corn using a ratio of 0.75:1 in a column extractor. Protein concentration and yields are higher in this case, possibly because they used deoiled corn, longer extraction times (6 hr), and lower solvent-to-solids ratio.

Continuous extraction was simulated by multiple extractions of the same batch of corn with fresh volumes of ethanol. This resulted in lower yields and zein concentrations in each extract (Fig. 8). However, the cumulative yield was  $\approx 85\%$  of the zein in the corn.

On the other hand, extracting fresh batches of corn with the same volume of ethanol resulted in an almost linear increase in zein concentration in the extract to 1.6% (w/v) after six extractions (Fig. 9). This is comparable to that observed in a packed column extractor at 1:1.

However, this increase in zein levels in the extractant decreased the zein-extraction capacity of the solvent with reuse, resulting in

lower yields in each stage. By the fourth reuse, only 20% of the zein in fresh corn could be extracted.

### Zein Content of Extracted Solids

The zein content of the extracted solids has seldom been addressed. It is expected that the ethanol or the water in the solvent would be simultaneously extracting nonzein components such as free fatty acids, lipids, free sugars, minerals, soluble starch, fiber, amino acids, carotenoids, and other pigments. Parris et al (1997) used SDS-capillary electrophoresis to identify various components in ethanol extracts of corn. These components have been referred to as fine corn particles (Dickey et al 1998), and are primarily removed by centrifugation (Dickey et al 1997), settling (Swallen 1940), membrane filtration (Kampen 1995), and similar clarification procedures. Substantial removal of these particles is essential to obtaining high purity zein.

In our experiments, zein content of the extracted solids was 20–60%. In batch extraction, zein content and yield of protein showed similar trends (Figs. 1–4). Zein content was constant at  $>30$  min of extraction time and at  $>50^\circ\text{C}$ . Maximum protein content was observed at 70% ethanol and a solvent-to-solids ratio of 8:1. It appeared that conditions that maximized extraction of zein minimized coextraction of nonzein components. With multiple extractions, reextracting the same corn with fresh ethanol solvent resulted in the highest zein content of 60% (db) after the second extraction (Fig. 8). However, there was a large drop in zein content thereafter due to the preferential extraction of nonzein components. On the other hand, using the same ethanol solvent for several extractions

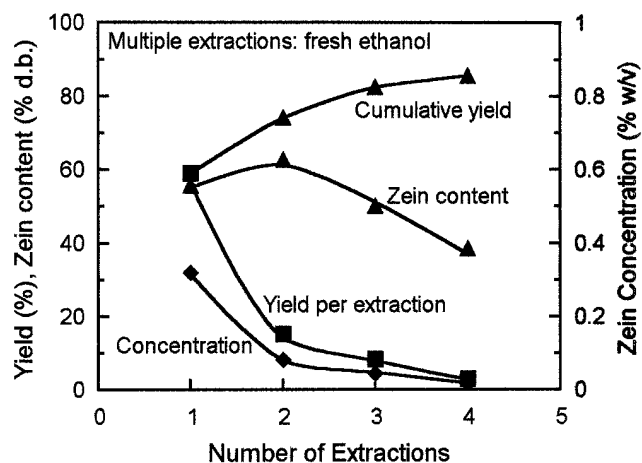


Fig. 8. Multiple extractions of corn. Corn recycle, fresh solvent in each extraction. Solvent-to-solids ratio 8:1, 70% ethanol, 30 min, 50°C.

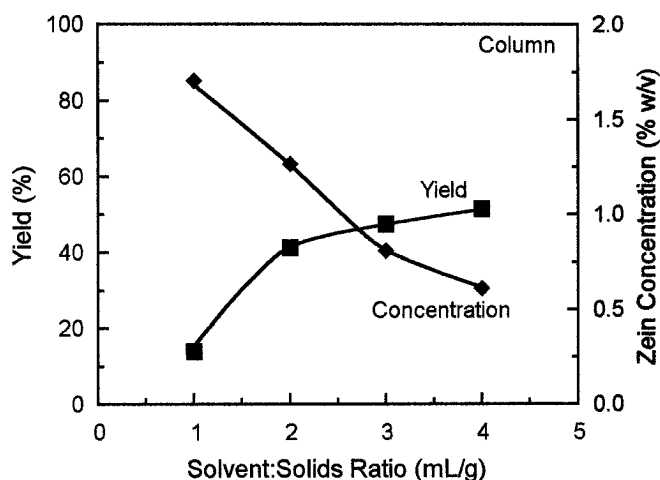


Fig. 7. Effect of solvent-to-solids ratio on column extraction of zein. 70% ethanol, 50°C, 25 min.

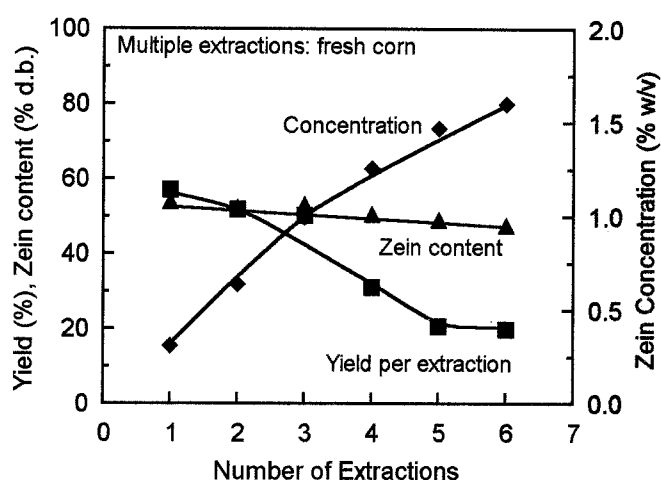


Fig. 9. Multiple extractions of corn. Ethanol recycle, fresh corn in each extraction. Solvent-to-solids ratio 8:1, 70% ethanol, 30 min, 50°C.

of corn, each time simulating a counter current extraction, had little effect on zein content (Fig. 9).

### Process Optimization

With batch extraction, the maximum protein extracted was with 70% ethanol with 45 min of extraction time at 50°C. Based on

these results, a 2<sup>4</sup> full-factorial design was constructed (Table II) as described above. Results from the quadratic model fitting and ANOVA of the resulting model (Shukla 2000) showed that temperature and solvent-to-solids ratio are the significant ( $P < 0.05$ ) effects. The curvature in the response surface is due to higher order effects of ethanol concentration and solvent-to-solids ratio

TABLE II  
Experimental Results

Design	Variables					Protein Content (g/L)
	Temperature (°C)	Ethanol (% v/v)	Ratio (mL/g)	Time (min)		
2 <sup>4</sup>	50	80	8	60		1.84
	50	80	8	30		1.75
	50	80	4	60		4.24
	50	60	8	60		2.61
	30	80	8	60		1.92
	50	80	4	30		3.54
	50	60	4	60		4.42
	30	60	8	60		1.56
	50	60	8	30		2.21
	50	60	4	30		3.81
	30	60	4	60		2.78
	30	60	8	30		1.43
	30	60	4	30		2.08
	30	80	8	30		1.33
	30	80	4	30		2.27
	Replicates at center	30	80	4	60	
40		70	6	45		2.50
40		70	6	45		2.82
40		70	6	45		2.77
40		70	6	45		2.93
Star coordinates	40	70	6	45		2.55
	40	70	6	45		2.95
	40	70	10	45		1.77
	40	70	2	45		6.59
	60	70	6	45		3.78
	40	90	6	45		1.77
	40	50	6	45		1.58
	20	70	6	45		1.40
40	70	6	75		3.10	
40	70	6	15		1.93	

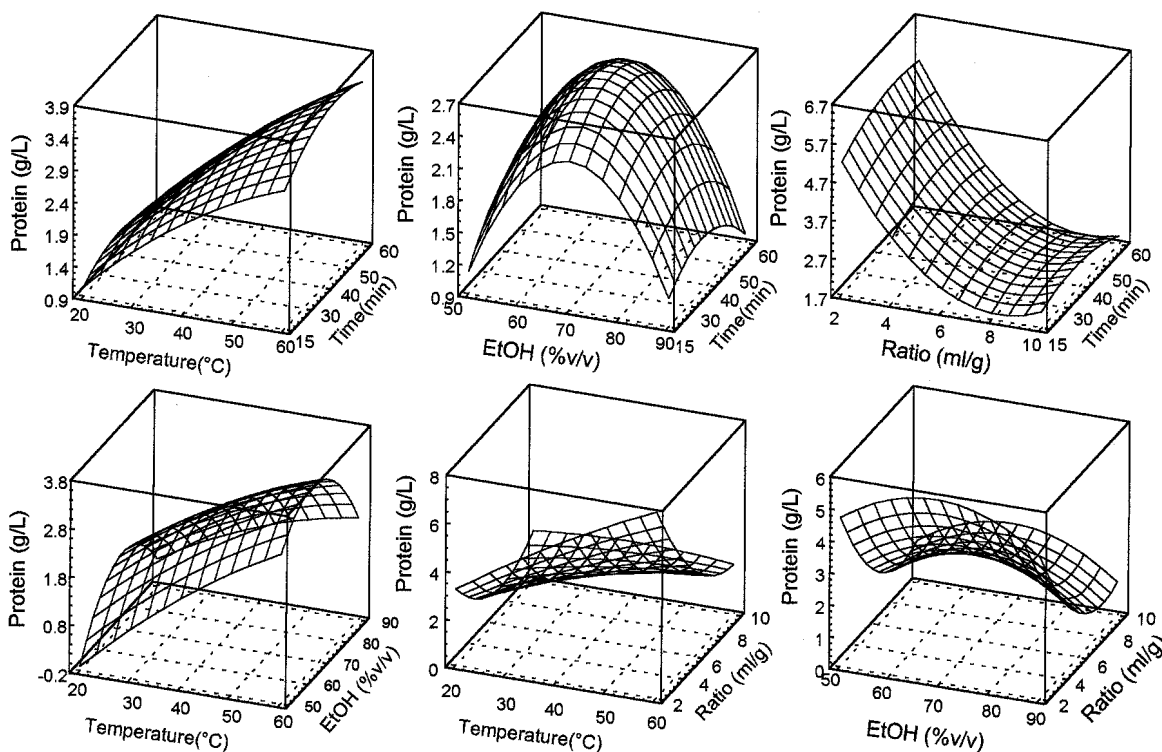
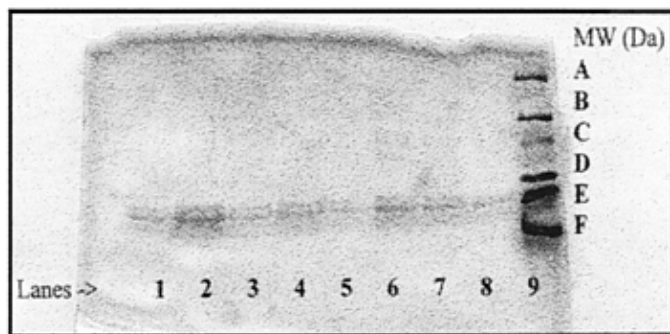


Fig. 10. Response surface plots for batch extraction of zein from corn.



**Fig. 11.** SDS-PAGE of ethanol extracts of corn and commercial zein produced using 70% ethanol, 50°C and 30 min of extraction time. Lanes 1 and 2: experimental samples with added 2 mM mercaptoethanol (ME); lane 3: commercial zein samples from Showa Sangyo zein + 2 mM ME; lane 4: commercial zein samples from Freeman (F4000) + 2 mM ME; lanes 5–8: same as lanes 1–4 without ME; lane 9: standard molecular weight markers: A = 97,400; B = 66,200; C = 45,000; D = 31,000; E = 21,500; F = 14,400.

and interactions of temperature and solvent-to-solids ratio (Shukla 2000). The significant effect of ethanol concentration was expected. Dill (1927) determined that maximum solubility of zein was at 70% ethanol concentration. This also explains the lack-of-fit obtained when a first-order model was used.

Response surface plots of the effect of different variables are shown in Fig. 10. They support the results obtained from the screening studies on the nature of the response for batch extraction of zein. The interaction of temperature and ratio is difficult to explain, it is not obvious from the curvature in the response surface plots. However, it points out the importance of studying not only the individual variables but also the interactions. The resulting quadratic model equation is:

$$\hat{Y} = 2.66 + 0.57x_1 + 0.84x_3 + 0.286x_1x_3 + 0.28x_2^2 + 0.33x_3^2 \quad (7)$$

By differentiating the quadratic model with respect to the variables, the location of the optimum conditions for extraction are:

$$\begin{bmatrix} \text{Temperature} \\ \text{Ethanol Concentration} \\ \text{Ratio} \\ \text{Time} \end{bmatrix} = \begin{bmatrix} 45.6 \text{ }^\circ\text{C} \\ 68.1\% \\ 7.8 \text{ ml/g} \\ 54.8 \text{ minutes} \end{bmatrix}$$

Canonical analysis indicated the optimum was a saddle point (i.e., neither a maximum nor a minimum). Response of protein concentration increased in the direction of lower solvent-to-solids ratio.

### Electrophoresis

SDS PAGE of ethanol extracts of corn was performed to compare our product with commercial zein products and to determine the size of the respective subunits (Fig. 11). All zeins showed two strong distinct bands at molecular weights (MW) of 22,000 and 24,000. A light band was also observed at MW of 44,000 when no reducing agent was used (Lanes 5–7). The band at MW 44,000 disappeared in the presence of the reducing agent (Lanes 1–3). This suggests that the two predominant bands are subunits joined by disulfide bonds.

### CONCLUSIONS

Optimum conditions for batch extraction of whole ground corn were determined with a quadratic model that described the protein response surface. The model was significant and described the process adequately. The optimum conditions for zein extraction were 45°C, 68% ethanol, solvent-to-solids ratio of 7.8 mL/g, and

an extraction time of 55 min. These values are close to the results obtained in screening studies (50°C, 70% ethanol, ratio of 8, and 30 min). A packed column extractor resulted in higher zein concentration in the extract with optimums at 50°C and 70% ethanol.

### ACKNOWLEDGMENTS

This research was supported by the Illinois Corn Marketing Board; Illinois Department of Commerce and Community Affairs, Bureau of Energy and Recycling; United States Department of Agriculture through the NRICGP program (Award No. 97-35504-4296) and the Illinois Agricultural Experiment Station. Analytical assistance was provided by Amanda D. Popp and Jason C. Friedrichs.

### LITERATURE CITED

- American Association of Cereal Chemists. 2000. Approved Methods of the AACC, 10th ed. The Association: St. Paul, MN.
- Anonymous. 1985. Wheat gluten, corn gluten, and zein; affirmation of GRAS status. Fed. Regist. 50:8997-8999.
- AOAC. 1984. Association of Analytical Chemists Official Methods of Analysis, 14th ed. The Association: Washington, D.C.
- Boudry, J. A., Turner, J. E., Wall, J. S., and Dimler, R. J. 1967. Influence of commercial processing on composition and properties of corn zein. *Cereal Chem.* 44:281-287.
- Box, G. E. P., Hunter, W. G., and Hunter, J. S. 1978. *Statistics for Experimenters*. John Wiley and Sons: New York.
- Chen, L., and Houston, D. F. 1970. Solubilization and recovery of protein from defatted rice bran. *Cereal Chem.* 47:72-79.
- Chen, L.-F., and Hoff, J. E. 1987. Grain extraction milling. U.S. patent 4,716,218.
- Cheryan, M. 1998. *Ultrafiltration and Microfiltration Handbook*. Technomic Publishing Co.: Lancaster, PA.
- Dickey, L., Dallmer, M. F., Radewonuk, E. R., Parris, N., Kurantz, M., and Craig, J. C. 1998. Zein batch extraction from dry-milled corn: Cereal disintegration by dissolving fluid shear. *Cereal Chem.* 75:443-448.
- Dickey, L. C., Dallmer, 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.
- Dickey, L. C., McAloon, A., Craig, J. C., and Parris, N. 1999. Estimating the cost of extracting cereal protein with ethanol. *Ind. Crops Prod.* 10:137-143.
- Dill, D. B. 1927. The behavior of the prolamins in mixed solvents. II. *J. Biol. Chem.* 72:239-247.
- Duvick, D. N. 1961. Protein granules of maize endosperm cells. *Cereal Chem.* 38:515-519.
- Hojilla-Evangelista, M. P. 1990. Sequential extraction process: Alternate technology for corn wet milling. PhD thesis. Iowa State University: Ames, IA.
- Hojilla-Evangelista, M. P., Johnson, L. A., and Myers, D. J. 1992. Sequential extraction processing of flaked whole corn: Alternative corn fractionation technology for ethanol production. *Cereal Chem.* 69:643-647.
- Kampen, W. H. 1995. Recovery of protein, protein isolate and/or starch from cereal grains. U.S. patent 5,410,021.
- Keim, C. R. 1995. Wet milling for grain alcohol production. Pages 1-10 in: *The Alcohol Textbook: Ethanol Production by Fermentation and Distillation*. T. P. Lyons, D. R. Kelsall, and J. E. Murtagh, eds. Nottingham University Press: Nottingham, UK.
- Lai, H. M., Padua, G. W., and Wei, L. S. 1997. Properties and microstructure of zein sheets plasticized with palmitic and stearic acids. *Cereal Chem.* 74:83-90.
- Lawhon, J. T. 1986. Process for recovery of protein from agricultural commodities prior to alcohol production. U.S. patent 4,624,805.
- Lending, C. R., and Larkins, B. A. 1989. Composition of protein bodies during maize endosperm development. *Plant Cell* 1:1011-1023.
- Lower, E. S. 1999. Zein of the times. *Spec. Chem.* 19:36-40.
- Mannheim, A., and Cheryan, M. 1993. Water-soluble zein by enzymatic modification in organic solvents. *Cereal Chem.* 70:115-121.
- McKinney, L. L. 1958. Zein. Pages 319-320 in: *The Encyclopedia of Chemistry (Suppl.)*. G. L. Clark, ed. Reinhold Publishing: New York.
- Mossé, J. 1961. Monographie sur une protéine du maïs: La zéine. (In French) *Ann. Physiol. Vég.* 3:105-139.
- Parris, N., Dickey, L., and Craig, J. 1997. Quantitative analysis of corn zein by capillary electrophoresis. *Cereal Chem.* 74:766-770.
- Reiners, R. A., Wall, J. S., and Inglett, G. E. 1973. Corn Proteins: Potential for their industrial use. Pages 285-302 in: *Industrial Uses of Cereals*.

- Y. Pomeranz, ed. *Am. Assoc. Cereal Chem.*: St. Paul, MN.
- Russell, M. H. 1980. Protein separation from corn endosperm by solvent extraction. PhD thesis. Purdue University: W. Lafayette, IN.
- Russell, M. H., and Tsao, G. T. 1982. Protein removal from corn endosperm by solvent extraction. *AIChE Symp. Ser.* 78(218):83-89.
- Shukla, R. 2000. Production of zein from dry milled corn by solvent extraction and ultrafiltration. PhD thesis. University of Illinois: Urbana-Champaign, IL.
- Swallen, L. C. 1940. Stabilization of a zein solution by clarification. U.S. patent 2,221,560.
- Turner, J. E., Boundy, J. A., and Dimler, R. J. 1965. Zein: A heterogeneous protein containing disulfide-linked aggregates. *Cereal Chem.* 42:453-461.
- Wu, Y. V., and Sexson, K. R. 1976. Protein concentrate from normal and high-lysine corns by alkaline extraction: Preparation. *J. Food Sci.* 41:509-511.

[Received January 21, 2000. Accepted August 15, 2000.]