Optimizing Extraction of Zein and Glutelin-Rich Fraction During Sequential Extraction Processing of Corn

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ABSTRACT

This study was conducted to improve yields and qualities of corn protein co-products produced by the sequential extraction process (SEP), a process using ethanol to fractionate corn in producing fuel ethanol. A two-stage extraction protocol was evaluated to recover zein and subsequently recover a glutelin-rich fraction (GRF). After the simultaneous oil-extraction and ethanol-drying step of SEP, zein was extracted from the anhydrous-ethanol-defatted, flaked corn by using 70% (v/v) ethanol at 60°C for 1.5 hr in a shaking water bath. Zein was recovered by ultrafiltering and then drying in a vacuum-oven. Zein yield was 65% of the available zein in the flaked corn. SDS-PAGE band patterns of the recovered zein closely resembled that of commercial zein. After zein extraction, the GRF was extracted using 45% ethanol and 55% 0.1*M* NaOH at 55°C for 2 hr. The extract was concentrated by ultrafiltration and then freeze-dried. GRF yield was ≈65% of the available protein. Freeze-dried GRF contained 90% crude protein (db), which classified the protein as a protein isolate. As with the protein concentrate from the original SEP, the GRF isolate was highly soluble in water at pH ≥ 7, had good emulsifying and foaming properties, formed stable emulsions, and was heat-stable.

The sequential extraction process (SEP) was developed to improve the profitability of producing fuel ethanol by producing new co-products with higher values than those achieved by traditional ethanol plants employing wet-milling or dry-grinding methods. SEP uses ethanol to extract oil from flaked, dried corn while simultaneously drying the alcohol in a countercurrent system and then extracting the protein from the defatted flakes using 45% ethanol and 55% 0.1*M* NaOH (Hojilla-Evangelista et al 1992b). Some zein was co-extracted with the crude corn oil. The freeze-dried protein extracted using ethanol and alkali from the corn contained >80% crude protein (db), which was significantly greater than the typical 60% protein content of corn gluten meal (Hojilla-Evangelista et al 1992a). SEP protein concentrate also had good functional properties (Myers et al 1994), especially solubility in water, and may be considered food-grade unlike the protein products from wet-milling, where the SO_2 steeping treatment relegates the protein to feed. Chang et al (1995) showed that SEP would generate a reasonable return in investment (20%) if a plant were sized for 20 million bushels per year (700,000 m^3/yr) of corn with an estimated capital investment cost of $225 million.

Despite demonstrating the technical feasibility of SEP and its potential to generate high-value co-products, the efficiency in terms of ethanol drying, protein recoveries, and fiber utilization require further improvement before industry will seriously consider SEP as a practical means of producing fuel ethanol. As a first step to achieving this goal, Miller et al (2002) examined the parameters for optimizing the oil-extraction and ethanol-drying step in SEP (i.e., minimizing zein co-extraction with the oil while increasing oil extraction from the corn and moisture adsorption from the ethanol). The optimum conditions involved extraction at 56°C with 30% hexanes and 70% ethanol in a single-pass mode of operation through the flake bed contained in an extraction column with a 15:1 L/D ratio (Miller et al 2002). Other factors (solvent-to-corn ratio, corn moisture content, and number of extraction stages) were also evaluated (Hojilla-Evangelista and Johnson 2002) for their effects on ethanol drying, oil recovery, and protein loss when the modified extraction system of Miller et al (2002) was used.

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Minimizing the amount of zein co-extracted with crude oil (Miller et al 2002) not only made oil recovery less complicated, but also achieved maximum zein recovery, which was accomplished by including another extraction step that was specific for this protein. The remaining glutelin-rich fraction (GRF) could be extracted as before by using ethanol and alkali and recovered by membrane filtration, which was used to recover corn protein extracted by ethanol (Lawhon 1986) and to deflavor soy protein isolate (Lawhon 1983). Recent work by Shukla and Cheryan (2002) evaluated the performance of ultrafiltration membranes with ethanol-water-protein solutions. The study was part of the efforts to develop a process for manufacturing zein wherein ultrafiltration was used to recover and purify zein while simultaneously recycling ethanol solvent.

The present work focuses on evaluating methods to improve the recoveries of zein and GRF from corn. These methods used separate extraction steps for zein and GRF and applied membrane filtration for protein recovery to reduce solvent evaporation costs. The effects of these process modifications on the compositional and functional properties of zein and GRF are also presented.

MATERIALS AND METHODS

Zein Extraction and Recovery

Two methods of zein extraction were evaluated. Method A was modified from the procedure of Carter and Reck (1970) and used by Wu (1996) to extract zein from corn gluten meal. Method B followed the conditions described by Swallen (1941) and was based on the procedure used by Hojilla-Evangelista et al (1992a) and Myers et al (1994) for extracting the GRF. Solvent concentration and extraction duration and temperature of method B were determined by preliminary experiments.

Method A. Zein was extracted from ground, dried, defatted, flaked corn by using 70% (v/v) aqueous ethanol. The solvent was added to the corn at a 4:1 (w/w) solvent-to-corn ratio. Extraction was conducted at 125 rpm for 1 hr in a shaking water bath maintained at 60°C. The extraction mixture was centrifuged for 15 min at 8,000 × *g* and room temperature, and then filtered through a Whatman no. 4 filter paper. The supernatant was chilled overnight at –18°C to precipitate the zein fraction, which was then redissolved in the extraction solvent and chilled overnight at –18°C to purify the zein. Precipitated zein was transferred into a tared weighing pan and its weight was recorded after evaporating the solvent.

Method B. Zein was extracted from ground, air-dried, defatted, flaked corn with a 4:1 (w/w) solvent-to-corn ratio of 70% (v/v) aqueous ethanol. Extraction was conducted at 125 rpm for 1.5 hr in a shaking water bath maintained at 60°C. The mixture was centrifuged for 10 min at 1,050 × *g* and room temperature. The

supernatant was concentrated through a 10-kDa regenerated cellulose membrane (Millipore Corp., Bedford, MA) by stirred-cell ultrafiltration (Amicon model 8400, Millipore Corp., Bedford, MA). Zein was recovered in solid form from the concentrated extract by air-drying to remove the ethanol, then by drying in a vacuum oven at 50°C and 0.6 mm Hg for 3–4 hr.

Appearances of the recovered zein were noted and mass yields were calculated. Purities of zein were determined by SDS-PAGE following the procedure described by Hojilla-Evangelista et al (1992a) and compared with that of commercial zein (94.7% crude protein content, db; Freeman Industries, Tuckahoe, NY).

Extraction and Recovery of Glutelin-Rich Fraction (GRF)

The GRF was extracted according to the procedure used by Hojilla-Evangelista et al (1992a) and Myers et al (1994). The solvent, 45% ethanol and 55% 0.1*M* NaOH, was added to the meal after extracting zein at 14:1 (v/w) solvent-to-corn ratio that was based on the dry weight of ground, defatted, flaked corn used at the start of zein extraction. Protein extraction was conducted at 125 rpm for 2 hr in a shaking water bath maintained at 55°C. The mixture was centrifuged for 10 min at 1,050 × *g* at room temperature. The supernatant was ultrafiltered at room temperature through a 10-kDa spiral-wound PLGC regenerated-cellulose membrane (Amicon model CH2PRS Ultrafiltration System, Millipore Corp., Bedford, MA). The pressure differential between the inlet and outlet was maintained at 10 psi and the flow rate was 60 mL/min. Ethanol and alkali were also removed from the extract by repeated additions of deionized water during membrane filtration before the concentration step. The volume of the retentate was reduced to ≈300 mL and then deionized water was added until the volume returned to the starting level of 1.2 L. The cycle was repeated five times, after which the retentate was concentrated to 100 mL. The concentrate was then freeze-dried to recover the GRF in solid form.

Moisture and crude protein contents of the freeze-dried GRF and the corn residue before and after GRF extraction were determined by Karl Fischer titration (ASTM 1975) and Kjeldahl methods (AACC 2000), respectively. SDS-PAGE was used to determine the types of proteins present based on approximate molecular weights of protein bands that were produced. Functional properties of GRF such as solubility (Myers et al 1994), heat coagulability (Myers et al 1994), gelation (Balmaceda et al 1984), foaming (Sorgentini et al 1995), and emulsification (Hung 1984) were also evaluated.

A substantial amount of the available zein was assumed to have been removed during the first protein extraction stage with only a small amount remaining for extraction by the ethanol and alkali solvent mixture, thus eliminating the need for the ethanol component of the extraction solvent. Therefore, another solvent, comprised only of 0.05*M* NaOH, was also evaluated for GRF extraction and the chemical and functional properties of the recovered protein solids were compared with those of GRF solids obtained by extracting with the ethanol and alkali solvent mixture.

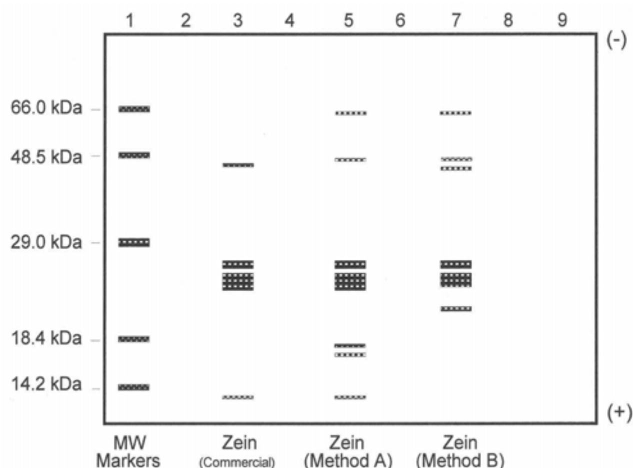

Fig. 1. Band patterns illustrating SDS-PAGE of commercial zein and zein recovered by chilling (method A) or ultrafiltering and vacuum-oven drying (method B).

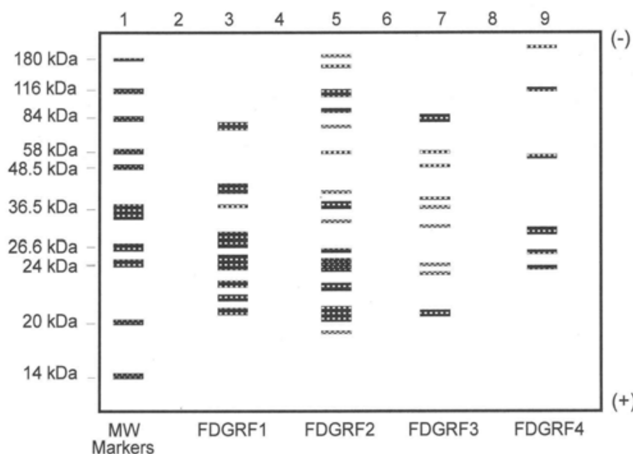

Fig. 2. Band patterns illustrating SDS-PAGE of glutelin-rich fractions extracted with ethanol and NaOH (FDGRF1); 0.05*M* NaOH (FDGRF2); ethanol and NaOH with additional soaking and grinding steps (FDGRF3); and 0.05*M* NaOH with additional soaking and grinding steps (FDGRF4).

Statistical Analyses

Statistical analysis was performed at the USDA-ARS National Center for Agricultural Utilization Research in Peoria, IL, by using the SAS Systems for Windows software (SAS Institute Inc., Cary, NC). Analysis of variance and *t*-test were used to determine significant differences among treatment means at $P \leq 0.05$.

RESULTS AND DISCUSSION

Zein Extraction and Recovery

Zein fractions produced by both methods had granular appearances and considerably lighter yellow color than did commercial zein, although zein from ultrafiltration and vacuum-oven drying (method B) was more yellow than zein precipitated by chilling (method A). Nearly 70% of the estimated zein available for extraction from defatted, flaked corn was recovered by method B (Table 1), a yield that was nearly three times greater than the 24% yield of zein achieved by chilling and that obtained by Wu (1996) from corn gluten meal. Zein from both methods revealed SDS-PAGE band patterns that closely resembled those of commercial zein; however, zein produced by method A (chilling) had more protein bands that did not correspond to other zein classes, indicating the presence of more impurities (Fig. 1). Purity of the recovered zein was improved when 80% (v/v) aqueous ethanol was used to extract the protein but the yield was reduced significantly (14% for method A and 44% for method B). Based on these results, the protocol that employed stirred-cell ultrafiltration and vacuum-oven drying was selected for zein extraction and recovery.

Extraction and Recovery of GRF

Both ethanol-NaOH and 0.05*M* NaOH extracted more than 40% of the available protein in the corn solids after zein extraction (Table II). However, the residual protein content in the fiber and starch residue was almost double that observed in an earlier SEP study (Hojilla-Evangelista et al 1992a,b), indicating lower protein extraction efficiencies for the method used in the present study. The freeze-dried GRF contained at least 85% crude protein (db), which classifies the protein products as protein concentrates (65–89% protein) (Table II).

SDS-PAGE band patterns showed two very different protein profiles for the GRF concentrates obtained by extracting with either ethanol-NaOH or 0.05*M* NaOH (Fig. 2). More protein bands were present in the GRF extracted with dilute alkali alone (FDGRF2) than in the protein sample extracted with the ethanol and alkali solvent mixture (FDGRF1). The band pattern of FDGRF2 was similar to those of corn glutelins (Wilson 1987). In FDGRF1, there were no protein bands corresponding to molecular weights (MW) > 85 kDa. The bands near the 24-kDa MW marker indicated the presence of zein, which was not surprising because the ethanol component of the solvent mixture can extract any remaining zein in the corn solids.

The difference in the protein classes present in FDGRF1 and FDGRF2 significantly affected the solubility profiles of the two protein concentrates (Fig. 3). The GRF concentrate generated by extraction using ethanol and alkali (FDGRF1) was more soluble than was the concentrate produced by extracting with alkali alone (FDGRF2). For FDGRF1, 70–85% of the protein remained soluble at pH ≥ 7, while for FDGRF2, only 60% of the protein remained soluble at the same pH range. The concentrate produced by extracting with 0.05*M* NaOH had better foaming capacity and stability than did the concentrate produced by extraction with ethanol and alkali (Table III). Emulsification capacities of both corn protein concentrates were markedly <800 g of oil/g of protein obtained from earlier SEP studies (Myers et al 1994). Dilute solutions of both concentrates were also very stable to heat (Table III).

Modification of Extraction Procedure for GRF

The high residual protein content after GRF extraction, low protein extraction efficiency, and poor solubilities and emulsifying capacities necessitated further improvements in the extraction procedure for GRF. To accomplish this goal, additional steps of soaking and grinding were incorporated in the procedure before the actual protein extraction step (Fig. 4). Soaking softened the corn particles for better solvent penetration while grinding increased the surface area contacting the solvent.

TABLE I
Yields and Protein Contents of Zein Extracted by Two Different Methods[a]

	Method A[b]	Method B[c]
Wt. of corn sample, g	25.0	25.0
Crude protein (CP) content, % (db)	8.62 ± 0.2	8.62 ± 0.2
Estimated zein content[d] (mg)	862	862
Amount of zein recovered (mg)	204 ± 20b	599 ± 53a
Estimated zein yield (%)	23.7 ± 2.3b	69.5 ± 6.1a
Fraction of CP extracted[e] (%)	9.4 ± 0.9b	27.1 ± 1.3a

[a] Values followed by the same letter in the same row are not significantly different ($P < 0.05$). Means with standard deviations for four replicates.
[b] Method A recovered zein by chilling and air-drying.
[c] Method B recovered zein by ultrafiltration and vacuum-oven drying.
[d] Calculated as wt of corn × CP × 0.40, where 0.40 represents the 40% accounted for by zein among protein classes in corn (Wilson 1987).
[e] Calculated as amount of zein recovered (g)/(wt of corn × CP).

TABLE II
Yield of Glutelin-Rich Fraction (GRF) Extracted by Ethanol and NaOH or 0.05*M* NaOH[a]

	Extraction Solvent	
	Ethanol-NaOH	0.05*M* NaOH
Crude protein (CP) content of corn solids after zein extraction (%, db)	7.79 ± 0.01b	7.92 ± 0.06a
CP content of fiber and starch residue after GRF extraction (% db)	4.42 ± 0.50a	4.32 ± 0.16a
Protein extracted (%)	43.3	45.4
CP content of freeze-dried GRF (%, db)	88.5 ± 0.5a	85.9 ± 0.5b

[a] Values followed by the same letter in the same row are not significantly different ($P < 0.05$). Means with standard deviations for three replicates.

These modifications markedly reduced residual protein content in the fiber-starch fraction and significantly increased protein extraction efficiency to >60% (Table IV). The freeze-dried GRF extracted by ethanol and alkali contained ≈90% crude protein (db), which classifies the protein as isolate. The protein content of the freeze-dried GRF extracted by 0.05*M* NaOH decreased from 86 to 76%, a result that contradicted the improved protein extraction efficiency.

SDS-PAGE band patterns revealed protein profiles for the GRF produced by the modified procedure that were very different from those described above, especially for GRF extracted by 0.05*M* NaOH (FDGRF4) (Fig. 2). The number of protein bands detected in the concentrate produced by extraction with alkali was substantially reduced when the modified procedure was employed.

Solubility profiles of the GRF from the modified procedure (FDGRF3 and FDGRF4) (Fig. 3) improved for the ethanol-alkali

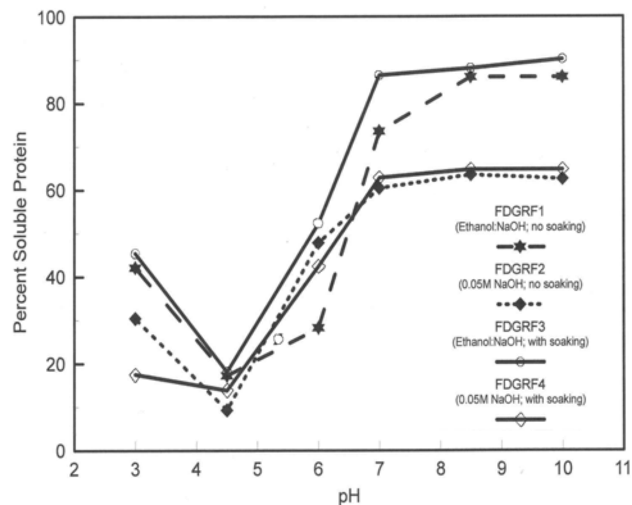

Fig. 3. Solubility profiles of glutelin-rich fractions extracted with ethanol and NaOH (FDGRF1); 0.05*M* NaOH (FDGRF2); ethanol and NaOH with additional soaking and grinding steps (FDGRF3); and 0.05*M* NaOH with additional soaking and grinding steps (FDGRF4).

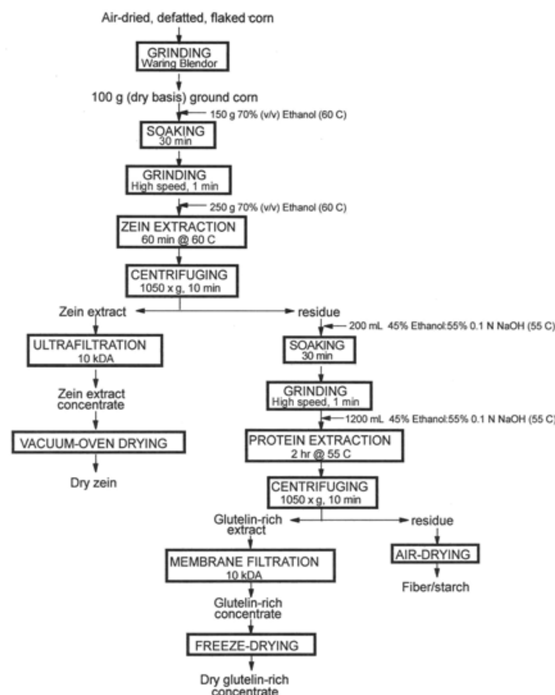

Fig. 4. Modified two-stage protocol for extracting zein and glutelin-rich fraction from corn.

GRF isolate only. More than 85% of the protein isolate remained soluble in water at pH ≥ 7. This degree of solubility more closely approached the results obtained by Myers et al (1994). The addition of soaking and grinding steps did not improve the solubility of the alkali-extracted GRF concentrate (Fig. 3). Only 60% of the protein concentrate still remained soluble in water at pH ≥ 7. Foaming capacities of both protein concentrates improved marginally, but foaming stabilities were slightly reduced (Table V). Emulsifying capacity of the ethanol and alkali-extracted GRF concentrate increased significantly, but no improvement was observed for that of alkali-extracted GRF concentrate (Table V). The protein solutions were also very heat-stable.

TABLE III
Selected Functional Properties of Concentrates of Glutelin-Rich Fraction (GRF) Extracted by Ethanol and NaOH or 0.05*M* NaOH[a]

	Extraction Solvent	
Functional Property	**Ethanol and NaOH**	**0.05*M* NaOH**
Foaming capacity[b]	1.37 ± 0.01a	1.43 ± 0.04a
Foam stability[c]	0.0162 ± 0.0009a	0.0114 ± 0.0004b
Emulsifying capacity[d]	601 ± 52a	542 ± 47b
Heat coagulability[e]	10.5 ± 0.8a	3.7 ± 2.2b

[a] Values followed by the same letter in the same row are not significantly different ($P < 0.05$). Means with standard deviations for three replicates.
[b] Calculated as FC = $V_f /(f_r \times t_f)$, where V_f is the fixed foam volume at the end of bubbling (150 mL); f_r is the flow rate of N_2 gas (100 mL/min); and t_f is the time (min) needed to reach V_f.
[c] Specific rate constant of drainage. Calculated as $k = 1/(V_{max} \times t_{1/2})$, where V_{max} is volume of liquid incorporated into foam at the end of bubbling (mL), and $t_{1/2}$ is time needed to drain half the liquid incorporated in foam (min).
[d] Expressed as g of oil/g of protein.
[e] Expressed as % loss in solubility.

TABLE IV
Yields of Glutelin-Rich Fraction (GRF) Extracted by Ethanol and NaOH or 0.05*M* NaOH Using a Modified Procedure with Additional Soaking and Grinding[a]

	Extraction Solvent	
	Ethanol and NaOH	**0.05*M* NaOH**
Crude protein (CP) content of corn solids after zein extraction (%, db)	6.94 ± 0.27a	7.08 ± 0.06a
CP content of fiber and starch residue after GRF extraction (%, db)	2.32 ± 0.06b	2.76 ± 0.06a
Protein extracted (%)	66.6	61.0
CP content of freeze-dried GRF (%, db)	89.9 ± 0.3a	76.0 ± 1.0b

[a] Values followed by the same letter in the same row are not significantly different ($P < 0.05$). Means with standard deviations for three replicates.

TABLE V
Selected Functional Properties of Concentrates of Glutelin-Rich Fraction (GRF) Extracted by Ethanol and NaOH or 0.05*M* NaOH Using a Modified Procedure with Additional Soaking and Grinding[a]

	Extraction Solvent	
Functional Property	**Ethanol and NaOH**	**0.05*M* NaOH**
Foaming capacity[b]	1.74 ± 0.01a	1.55 ± 0.06b
Foam stability[c]	0.0307 ± 0.0019a	0.0216 ± 0.0015b
Emulsifying capacity[d]	823 ± 25a	538 ± 22b
Heat coagulability[e]	3.0 ± 2.0a	2.5 ± 0.0a

[a] Values followed by the same letter in the same row are not significantly different ($P < 0.05$). Means of three replicates
[b] Calculated as FC = $V_f /(f_r \times t_f)$, where V_f is the fixed foam volume at the end of bubbling (150 mL); f_r is the flow rate of N_2 gas (100 mL/min); and t_f is the time (min) needed to reach V_f.
[c] Specific rate constant of drainage. Calculated as $k = 1/(V_{max} \times t_{1/2})$, where V_{max} is the volume of liquid incorporated into foam at the end of bubbling (mL) and $t_{1/2}$ is time needed to drain half the liquid incorporated in the foam (min).
[d] Expressed as g of oil/g of protein.
[e] Expressed as % loss in solubility.

CONCLUSIONS

Using 45% ethanol and 55% 0.1*M* NaOH in the modified procedure for GRF extraction achieved higher protein extraction efficiency and produced a corn protein isolate that possessed more attractive functional properties compared with those observed for the concentrate produced by extracting with only 0.05*M* NaOH. These results also demonstrated the technical feasibility of using two separate stages for extracting zein, and then the glutelins and albumins in the GRF.

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