

Isolation and Characterization of Zein from Corn Distillers' Grains and Related Fractions

WALTER J. WOLF^{1,2} and JOHN W. LAWTON, JR.¹

ABSTRACT

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Corn distillers' grains with solubles (CDGS), the major coproduct of fermentation of corn to produce ethanol, were extracted with 0.1M NaOH, 0.1% dithiothreitol (DTT), and 0.5% SDS yielding 35% of the total nitrogen and \approx 25% of the protein nitrogen. Gel electrophoresis revealed that the extractable proteins contained zein plus other proteins similar to the extractable proteins from corn flour. Although difficult to extract, the proteins isolated from the fermentation coproducts appeared undegraded and apparently survived gelatinization, fermentation, distillation, and drying during the production of ethanol. Extraction of CDGS with 60% ethanol at 60°C yielded 1.5-3.9% of crude zein. When

the ethanol contained DTT, yields of crude zein were increased to 3.2-6.6%. Protein contents of the crude zeins were only 37-57%, indicating that lipids and pigments were coextracted with the ethanol. Gel electrophoresis showed that the protein fractions extracted by ethanol contained primarily α -zein whereas the proteins extracted by ethanol + DTT contained α - + β -zein. Further confirmation of the presence of zein in the crude prolamin preparations was obtained by amino acid analyses. The amino acid compositions of the crude zeins paralleled those of commercial zein and α -zein.

Corn is the primary raw material used in the United States for the production of ethanol by fermentation. After fermentation, the resulting beer is distilled to yield ethanol and stillage, a dilute slurry containing 5-10% solids consisting of soluble and insoluble materials. The stillage solids represent 29-35% of the starting corn (Wu 1989) and their recovery is essentially a dewatering process. Typically, the stillage is screened or centrifuged to recover the insoluble material, the corn distillers' grains (CDG). The soluble fraction, corn distillers' solubles (CDS), is usually evaporated to a syrup, which may be marketed as is or, more commonly, may be added back to the wet CDG and drum-dried to yield corn distillers' grains with solubles (CDGS). Protein contents of CDGS are reported to range from 29 to 33%, depending upon the type of corn used, but dent corn is the only kind of corn used commercially, and yields CDGS with protein contents of \approx 33% (Wu 1989).

The primary use for CDGS is as feed for livestock, especially dairy cattle. CDG and CDGS have been evaluated as potential protein supplements in a variety of foods including bread (Satterlee et al 1976, Tsen et al 1983), cookies (Tsen et al 1982), wheat muffins (Reddy et al 1986b), blended foods for overseas feeding operations (Bookwalter et al 1984, Wall et al 1984), canned meat products (Reddy et al 1986a), and spaghetti (Wu et al 1987). Major problems for food use of the fermentation coproducts are the unacceptable fermented flavor and the deficiency of lysine (Bookwalter et al 1984).

Another potential application of CDGS is the formulation of aquaculture diets for species such as catfish and tilapia (Wu et al 1994, Sessa and Wu 1996). CDGS are utilized as well as a commercial control diet.

At present there are no known industrial uses for CDG and CDGS. Recovery of the chemical coproducts, glycerine and succinic acid, from stillage has been considered (Cygnowicz-Provost and Shapouri 1994) but is not being practiced. The possibility

of utilizing the functional properties of the residual proteins in CDG and CDGS in industrial products does not appear to have been reported.

Zein constitutes about one-half of the protein in corn (Wilson 1987) and likely is also the major protein in CDGS, although it may possibly be in a modified form because of the effects of processing. Zein is very hydrophobic and soluble in alcohol. The hydrophobicity of zein suggests its possible use, preferably in the crude form of CDGS, with biodegradable polymers such as starch to impart water resistance. Although CDGS contain 29-33% crude protein ($N \times 6.25$) (Wu 1989), this protein requires comparatively drastic solvents to solubilize it (Satterlee et al 1976, Wu et al 1981). For example, Satterlee et al (1976) used sodium hydroxide at pH 12 and extracted 54-56% of the protein when the extraction was done at 80°C. However, the extracted proteins were not identified and their amino acid composition was similar to that of the starting corn. Wu et al (1981) found that extraction of CDGS with 70% ethanol yielded only \approx 2% of the protein compared to \approx 40% from unfermented corn. The ethanol-extractable fraction was low in lysine as expected for zein, but the fraction was not characterized further. The low protein solubility was attributed to denaturation during distillation of the alcohol after fermentation. By using the more drastic conditions of NaOH + reducing agent + detergent, Wu et al (1981) were able to solubilize \approx 80% of the total protein.

TABLE I
Sources, Protein, and Nonprotein Nitrogen (NPN) Contents of Samples

Sample ^a	Source ^b	Protein (% db)	NPN (%)
CF	NCAUR	9.2	6.3 \pm 0.2
CGM-AD	PE	73.1	3.4 \pm 0.3
CGM-CD	PE	68.7	3.7 \pm 0.3
WS-FD	ADM	31.5	21.9 \pm 0.3
SC-FD	ADM	33.7	3.9 \pm 0.7
CDGS-1	ADM	29.5	3.7 \pm 0.6
CDGS-2	ADM	27.7	11.4 \pm 0.2
CDGS-3	B-F	27.1	3.4 \pm 0.2
CDGS-4	B-F	27.8	...

^a Corn flour (CF); corn gluten meal air-dried (CGM-AD) and commercially dried (CGM-CD); whole stillage freeze-dried (WS-FD); Sharples cake freeze-dried (SC-FD); distillers' grains with solubles freeze-dried (CDGS-1) and commercially dried (CDGS-2, CDGS-3, CDGS-4).

^b NCAUR = National Center for Agricultural Utilization Research, PE = Pekin Energy Co., ADM = Archer Daniels Midland, B-F = Brown-Forman.

¹Plant Polymer Research, National Center for Agricultural Utilization Research, USDA, ARS, Peoria, IL 61604. Mention of firm names or trade products does not imply that they are endorsed or recommended by the USDA over other firms or similar products not mentioned.

²Corresponding author. E-mail: wolfwj@mail.ncaur.usda.gov

The purpose of this study was to determine whether zein survives the fermentation, distillation, and drying steps in the processing of CDGS and to quantitate and characterize any residual zein that is recoverable from CDGS.

MATERIALS AND METHODS

Samples

Coarsely ground commercial yellow dent corn was obtained from Y. V. Wu and B. D. Deadmond of this Center and ground into a flour in an analytical micromill (Chemical Rubber Co., Cleveland, OH). Two corn gluten meal samples were obtained from Pekin Energy Co. (Pekin, IL). One was recovered from the Sharples centrifuge and air-dried at our Center and the other was dried commercially. Samples of whole stillage, Sharples centrifuge cake (CDG), and CDGS before and after commercial drying, were obtained from a local distillery of Archer Daniels Midland Co.. The whole stillage, Sharples cake, and wet CDGS samples were freeze-dried at our Center. Additional samples of CDGS were obtained from Brown-Forman Corp. (Louisville, KY). Nitrogen was determined by the Kjeldahl method (AOAC 1990).

Extracting Proteins from CDGS and Corn Flour

The proteins from CDGS were isolated for gel electrophoretic analysis by using the most robust solvent described by Wu et al (1981) in their studies of the extractability of the proteins. CDGS-2 (Table I) (2 g) was extracted with 20 mL of 0.1M NaOH, 0.1% dithiothreitol (DTT), and 0.5% SDS for 30 min and then centrifuged. The pellet was reextracted twice with 10 mL of the same solvent. The extracts were combined, analyzed for Kjeldahl nitrogen, and the remainder was dialyzed against distilled water and then freeze-dried. A similar experiment was performed with corn flour as a control.

Preparation of α -, β -, and γ -Zeins

The three zeins were isolated by the procedure of Esen (1986) with minor modifications. Crude zein was extracted from 50 g of

corn flour by magnetic stirring for 2 hr with 500 mL of 60% 2-propanol and 1% 2-mercaptoethanol, followed by reextraction with 250 mL of the same solvent for 1 hr instead of using four extractions as suggested by Esen. The rest of the fractionation followed that of Esen and yielded 165 mg of α -zein, 19 mg of β -zein, and 9 mg of γ -zein (freeze-dried, uncorrected for moisture). The isolated zeins represented 44% of the total protein ($N \times 6.25$).

Gel Electrophoresis

SDS-PAGE was conducted according to the Fling and Gregerson (1986) modification of the Laemmli (1970) method. A model SE 600 electrophoresis cell (Hoefer Scientific Instruments, San Francisco) was used. Gels were prepared with AcrylAide (FMC BioProducts, Rockland, ME) in place of *N,N'*-methylenebis (acrylamide) as the cross-linking agent. Gel compositions were 13.5% total polyacrylamide (T) and 0.4% cross-linker (C). The gels were stained with Coomassie Blue (Fling and Gregerson 1986). Molecular weight standards used were a broad range kit consisting of myosin, β -galactosidase, phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, lysozyme, and aprotinin (BioRad Laboratories, Hercules, CA).

Nonprotein Nitrogen Analyses

Extractability of nitrogen as a function of trichloroacetic acid (TCA) concentration was conducted as for jojoba, soybean, and almond meals (Wolf et al 1994, Wolf 1995), except that samples of 500–1,000 mg were used per 10 mL of TCA solution because of the low nitrogen and nonprotein nitrogen (NPN) contents of some of the samples. Minimum extractability of nitrogenous compounds in TCA was assumed to represent the NPN.

Extraction of Zein

Samples were extracted with 60% ethanol at 60°C (Swallen 1941) without and with 0.1% DTT. Three extractions were made: 10:1 solvent to sample for 30 min followed by two extractions with 5:1 solvent to sample. The volume of each extract was meas-

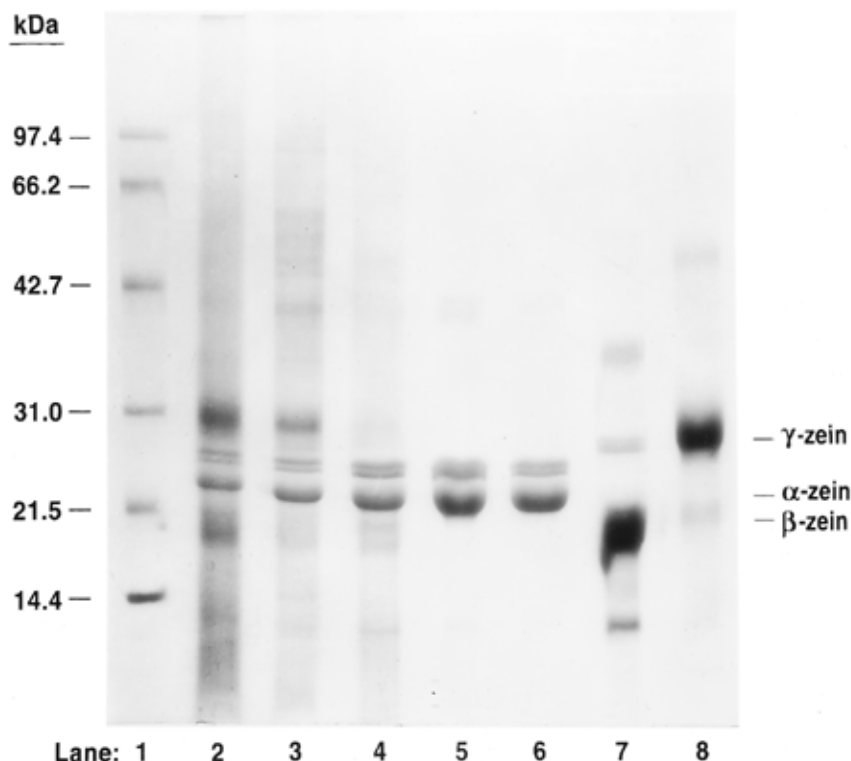


Fig. 1. Polyacrylamide gel electrophoresis of the proteins extracted from commercially dried distillers' grains with solubles (CDGS-2), corn flour (CF) and corn gluten meal (CGM). Lane 1: molecular weight standards; lane 2: CDGS-2 proteins; lane 3: CF proteins; and lane 4: CGM proteins. Reference proteins are lane 5: commercial zein; lane 6: α -zein; lane 7: β -zein; and lane 8: γ -zein.

ured, samples were removed for Kjeldahl analysis to measure the amount of nitrogen extracted, and the remaining extracts were then dialyzed separately against distilled water at 4°C to remove the ethanol. After dialyzing for several days, the precipitated proteins were freeze-dried and weighed. Yields were calculated with corrections for the volumes of extracts removed for nitrogen analysis.

Amino Acid Analyses

Analyses of freeze-dried ethanol-extractable proteins were conducted by the Experiment Station Chemical Laboratories, University of Missouri-Columbia, Columbia, MO using AOAC (1990) methods. The analyses included cysteine, methionine, and tryptophan.

RESULTS AND DISCUSSION

Although CDGS were of primary interest in this study, corn flour, corn gluten meal, and several fractions closely related to CDGS were included for comparative purposes. Sources, protein

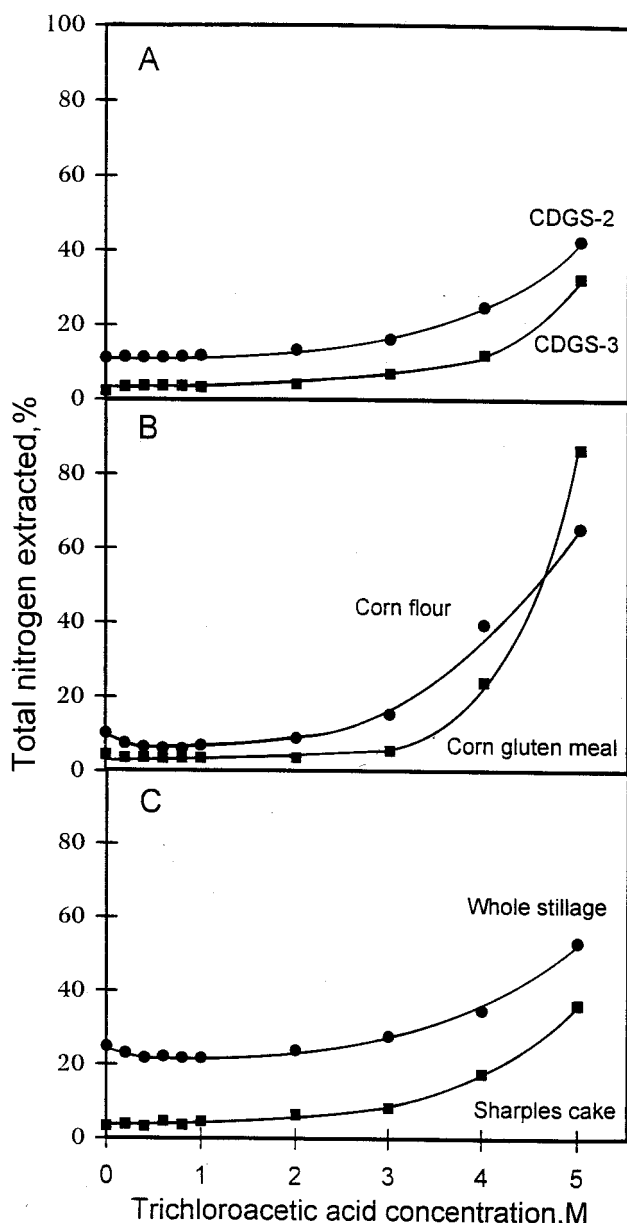


Fig. 2. Extractability of nitrogen as a function of trichloroacetic acid concentration. A, Commercially dried distillers' grains with solubles (CDGS-2 and CDGS-3). B, Corn flour and commercially dried corn gluten meal. C, Whole stillage and Sharples cake.

contents, and NPN contents of the various samples are summarized in Table I.

Gel Electrophoresis of Proteins Extractable from CDGS

Gel electrophoresis was employed to determine whether zein and the other corn proteins survived the processing (gelatinization of starch, fermentation, distillation, and drying [see Maisch 1987]) encountered in the manufacturing of CDGS. Initial experiments confirmed that the proteins were difficult to extract from CDGS as observed by Satterlee et al (1976) and Wu et al (1981). Using 0.1M NaOH, 0.1% DTT, and 0.5% SDS as the solvent, we attempted to extract the total protein but were able to extract only 35% of the nitrogen from CDGS-2 (see Table I for identification of sample) as compared to 91% of the nitrogen from corn flour. Analysis by SDS-PAGE (Fig. 1) revealed that the extracted proteins from CDGS-2 (lane 2) closely resembled the proteins extracted from corn flour (lane 3) with the same solvent. The proteins from corn gluten meal (lane 4), commercial zein (lane 5), α -zein (lane 6), β -zein (lane 7), and γ -zein (lane 8) were also run for comparison. The extractable proteins from CDGS-2 consist primarily of α -, β - and γ -zein, plus higher and lower molecular weight fractions found in corn flour. Corn gluten meal proteins and commercial zein are principally α -zein. The results show that the extractable proteins of CDGS-2 survived the processing steps but drastic extraction conditions are needed to solubilize them. The absence of low molecular weight bands that did not match those in corn flour suggests that little degradation (e.g., proteolysis) occurred during the fermentation. Presumably, the unextractable proteins are also undegraded but highly aggregated as a result of the heat treatment during processing.

NPN Content of CDGS and Related Fractions

Although Kjeldahl analysis indicated that 35% of the nitrogen of DCGS-2 was extractable with NaOH, DTT, and SDS, it was not known how much of the nitrogen originated from the NPN in the starting corn and from degradation that may have occurred during fermentation and subsequent processing. We therefore estimated the NPN by extraction with TCA. Because the optimal concentration of TCA to use was not found in the literature, samples were extracted with 0–5M TCA, and the minimal values of nitrogen extractability were assumed to represent NPN as previously demonstrated with jojoba (Wolf et al 1994) and soybean and almond meals (Wolf 1995). Figure 2A shows extractability curves for two samples of CDGS. The extractability curves are typical of materials in which the proteins are denatured and nitrogen solubility is constant from 0 to 1M TCA. Although the proteins are denatured, they are solubilized at high concentrations (1–5M) of TCA, as was observed for other protein meals (Wolf et al 1994, Wolf 1995). From the horizontal portion of the curves, the NPN value for CDGS-2 was estimated at 11.4%, whereas the value for CDGS-3 was considerably lower at 3.4%. From these results it is apparent that approximately one-third of the nitrogen extracted from CDGS-2 with NaOH, DTT, and SDS was NPN, thus only \approx 25% of the protein was extracted.

Also shown in Fig. 2 for comparative purposes are extractability curves for corn flour and commercially dried corn gluten meal (Fig. 2B), plus curves for whole stillage and Sharples cake (Fig. 2C). The extractability curve for corn flour indicates that 10.2% of the nitrogen was soluble in water (0M TCA) and represents the albumins + NPN. At 0.4–0.8M TCA, the solubility decreased to 6.3%, which is assumed to be the NPN fraction, thus the albumin content is \approx 4%. These values compare with averages of 6 and 7%, respectively, for albumins and NPN in whole corn kernels (Wilson 1987). Commercially dried corn gluten meal had a NPN content of only 3.7%, which is not surprising because it is prepared by wet-milling and is dewatered to 55–60% moisture before drying. NPN would therefore tend to be leached out (May 1987). Air-dried corn gluten meal had a similar value of 3.4% NPN (Table I).

Whole stillage exhibited an extractability curve (Fig. 2C) that suggested the presence of a small amount of protein that was soluble at 0M TCA but which precipitated at 0.4–1.0M TCA; the corresponding NPN content was 21.9%. This value is high compared to the values for the CDGS samples (Fig. 2A and Table I), which are essentially whole stillage after drying. Sharples cake, which is prepared by centrifuging whole stillage, exhibited an extractability curve that suggested the absence of soluble protein and a low NPN value of 3.9% (Fig. 2C). Clearly, the NPN is concentrated in the solubles. A portion of the NPN consists of amino acids, particularly alanine, and free sugars are also present (Dowd et al 1993). It is therefore likely that part of the NPN is polymerized in the form of browning reaction products during the concentration of the solubles to the syrup stage and during the final drying of the CDGS. Such browning reactions may account for the low NPN values for CDGS as compared to the high NPN content of whole stillage. The NPN values for the various samples are summarized in Table I.

Extraction of Zein

The results of SDS-PAGE indicated that zein was still present in CDGS. We therefore extracted CDGS and related fractions with 60% ethanol at 60°C (Swallen 1941) without and with reducing agent (0.1% DTT) to estimate the amount of extractable zein. As shown later, the proteins solubilized in the presence of DTT are primarily α - and β -zein. Hence, the proteins extracted in the absence and presence of reducing agent are simply referred to as zein. Table II shows percent of total nitrogen extracted and yields of crude zein obtained from the various samples. The overall averages for nitrogen extracted and yield were higher with ethanol + DTT ($P < 0.01$ by analysis of variance [ANOVA]) than with simple ethanol extraction. Although the amount of the increase varied in every case, there was an increase. Starting with corn flour, we extracted 27.0% of the nitrogen with ethanol and 42.5% with ethanol + DTT. The increased extraction with DTT reflects the effect of cleaving disulfide bonds of the classical glutelin fraction, which is insoluble in alcohol in the absence of a reducing agent. This fraction has been referred to by a variety of names including glutelin-1 (Landry and Moureaux 1970), zein-2 (Sodek and Wilson 1971), and alcohol-soluble reduced glutelin (Paulis and Wall 1977). Hamaker et al (1995) consider it to be a portion of the total zein. Our yields of crude zein from flour were 3.2–4.3% for the two extraction conditions. Air-dried corn gluten meal yielded 66.4% of the meal nitrogen in the ethanol extracts, but the yield of nitrogen increased to only 72.1% when DTT was included. The smaller effect of DTT as compared to results with corn flour suggest that many of the disulfide bonds linking the alcohol-insoluble proteins had been cleaved during the sulfur dioxide steeping step in wet-milling (Neumann et al 1984, May

1987). Approximately one-half of the corn gluten meal was recovered as crude zein when the extracts were dialyzed and freeze-dried. Results with commercially dried corn gluten meal were somewhat lower than with the air-dried meal, possibly because of protein-carbohydrate interactions that may have occurred during the drying. The commercially dried sample was noticeably darker in color than the air-dried sample, suggesting that browning reactions took place during drying.

Extraction of freeze-dried whole stillage resulted in removal of almost 26% of the nitrogen, and inclusion of DTT increased extractability of nitrogen to 32%. The high extraction of nitrogen is probably a reflection of the high NPN of this fraction (Fig. 2C). The high NPN content would also account for the low yield of crude zein (≈ 4 –6%) from whole stillage irrespective of the absence or presence of DTT. Inclusion of DTT in the ethanol had a marked effect on the extractability of nitrogen from the Sharples cake and this was also reflected in the yield of crude zein (12%) obtained by freeze-drying. Extraction of freeze-dried CDGS (CDGS-1) with DTT present resulted in higher yields of nitrogen and crude zein as compared to extraction in the absence of reducing agent. Similar effects were noted with the commercially dried samples (CDGS-2, CDGS-3, and CDGS-4). Yields of extractable nitrogen ranged from 4–19% in the absence of DTT and increased to 18–28% in its presence. Corresponding yields of crude zein ranged from 2–4% and increased to 3–6% when reducing agent was included. These yields of zein are on the same order as those noted for corn flour.

Attempts were also made to extract zein from CDGS-2 and CDGS-3 with 88% 2-propanol and 0.25% NaOH (Carter and Reck 1970), but they were unsatisfactory because of difficulties with lipids in the extracts. Centrifugation of the dialyzed 2-propanol extracts yielded floating lipids, and freeze-drying of the precipitates resulted in sticky residues of zein and lipids that made quantitation very difficult.

SDS-PAGE of Crude Zein Isolates

Extractability into ethanol suggested that the materials isolated from corn flour and the various processed fractions are zein. This was confirmed by SDS-PAGE. Figure 3 shows representative samples of crude zein obtained by extraction with 60% ethanol (without DTT). Zein isolated from corn flour (lane 2) consisted primarily of α -zein, whereas the material isolated from corn gluten meal (lane 3) also contained β -zein. Zein isolates from whole stillage (lane 4), Sharples cake (lane 5), CDGS-1 (lane 6), and CDGS-3 (lane 7) were very similar and consisted mainly of α -zein. Commercial zein (lane 8) contained primarily α -zein and, contrary to our isolate from corn gluten meal, did not contain any β -zein. Indeed, the isolates from stillage, Sharples cake, and CDGS closely resembled commercial zein. Lanes 9 and 10 were isolated α - and β -zein, respectively.

TABLE II
Yield of Zein Extracted at 60°C^a

Starting Material ^b	Source ^c	Nitrogen Extracted (%)		Freeze-Dried Yield (%)	
		-DTT ^d	+DTT	-DTT	+DTT
CF	NCAUR	27.0	42.5	3.2	4.3
CGM-AD	PE	66.4	72.1	49.8	52.0
CGM-CD	PE	61.6	69.0	43.9	47.9
WS-FD	ADM	25.9	32.3	4.0	5.6
SC-FD	ADM	8.3	32.0	4.2	12.3
CDGS-1	ADM	17.8	27.8	3.9	6.6
CDGS-2	ADM	17.4	27.5	3.8	4.7
CDGS-3	B-F	4.1	17.9	1.5	5.5
CDGS-4	B-F	18.9	25.0	2.1	3.2

^a Values are means of two or three analyses.

^b Definitions: corn flour (CF); corn gluten meal air-dried (CGM-AD) and commercially dried (CGM-CD); whole stillage freeze-dried (WS-FD); Sharples cake freeze-dried (SC-FD); distillers' grains with solubles freeze-dried (CDGS-1) and commercially dried (CDGS-2, CDGS-3, CDGS-4).

^c NCAUR = National Center for Agricultural Utilization Research, PE = Pekin Energy Co., ADM = Archer Daniels Midland, B-F = Brown-Forman.

^d Dithiothreitol.

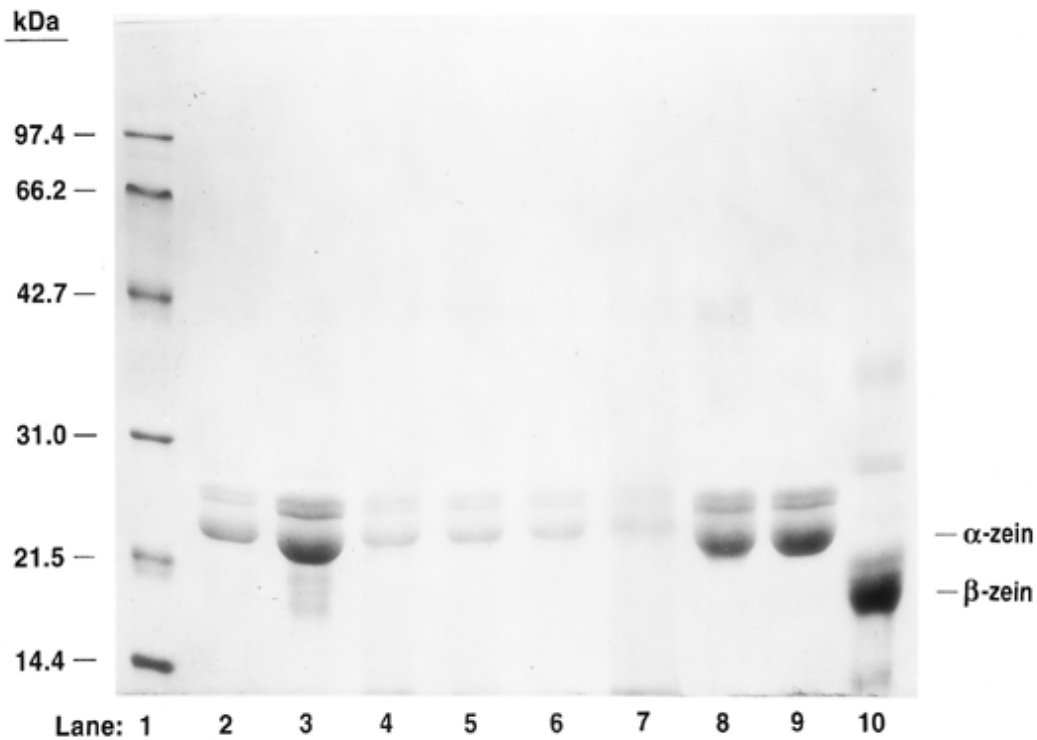


Fig. 3. Polyacrylamide gel electrophoresis of crude zeins extracted from various samples by 60% ethanol. Lane 1: molecular weight standards; lane 2: corn flour; lane 3: corn gluten meal; lane 4: whole stillage; lane 5: Sharples cake; lanes 6 and 7: commercially dried distillers' grains with solubles CDGS-2 and CDGS-4. Reference proteins are lane 8: commercial zein; lane 9: α -zein; and lane 10: β -zein.

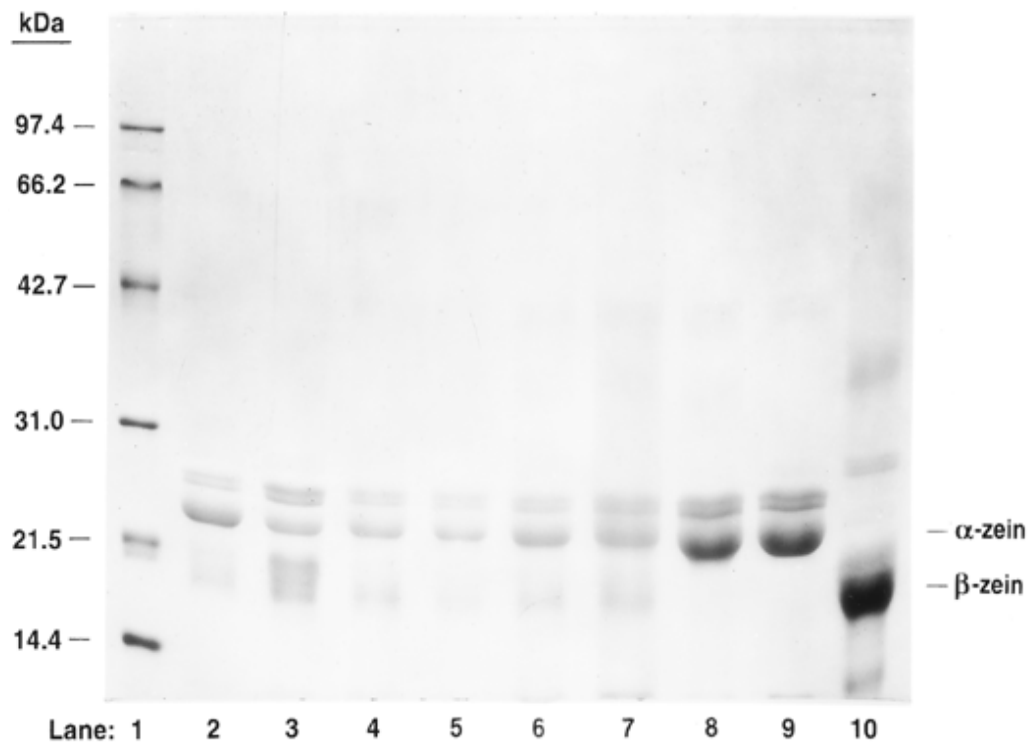


Fig. 4. Polyacrylamide gel electrophoresis of crude zeins extracted from various samples by 60% ethanol + dithiothreitol (DTT). Lane 1: molecular weight standards; lane 2: corn flour; lane 3: corn gluten meal; lane 4: whole stillage; lane 5: Sharples cake; lanes 6 and 7: commercially dried distillers' grains with solubles CDGS-2 and CDGS-4. Reference proteins are lane 8: commercial zein; lane 9: α -zein; and lane 10: β -zein.

The zein isolates obtained by extraction with ethanol + DTT were very similar to those obtained in the absence of DTT, except for the presence of small amounts of β -zein (Fig. 4). These small amounts of β -zein do not appear to account for the increased yields of total zein obtained with ethanol + DTT in the case of the Sharples cake where a threefold increase in yield was noted (Table

II); the reducing agent must also have increased the extractability of α -zein.

Amino Acid Composition of Crude Zein Isolates

Further confirmation of the identity of the crude isolates as zein was obtained by amino acid analysis. Table III shows protein

TABLE III
Protein Contents and Amino Acid Composition of Zein Isolates, Commercial Zein, α -, and β -Zein

Constituent ^{a,b}	Source of Isolate ^c								Zein		
	CF		CGM-CD		CDGS-1		CDGS-3		Commercial	α	β
	-DTT ^d	+DTT	-DTT	+DTT	-DTT	+DTT	-DTT	+DTT			
Protein	58.4	68.7	86.7	84.7	48.0	56.6	36.6	36.7	94.8	35.5	102.9
Asp	5.94	5.94	5.84	5.95	5.38	5.69	6.07	7.02	5.98	5.86	1.12
Thr	3.12	3.06	3.10	3.23	2.72	3.12	3.09	4.04	2.98	3.15	2.81
Ser	6.03	5.83	5.97	5.86	4.07	5.42	4.44	5.68	5.29	5.72	3.33
Glu	27.02	23.72	29.46	30.01	20.09	25.17	20.58	27.84	29.81	26.54	21.18
Pro	10.76	10.82	10.53	10.84	7.69	10.37	9.04	11.87	10.83	12.07	12.31
Gly	1.49	1.96	1.43	1.70	1.56	2.12	2.58	3.58	1.33	1.56	4.68
Ala	11.13	10.14	10.64	11.10	8.81	10.37	7.27	11.44	10.64	10.77	5.68
Cys	1.09	1.73	1.24	1.52	0.80	1.52	1.20	2.47	0.76	1.39	4.58
Val	3.97	3.48	3.71	4.13	3.88	4.18	3.81	5.13	4.25	3.96	3.35
Met	1.69	2.88	1.85	2.22	1.29	2.76	1.43	4.10	1.70	1.76	2.54
Ile	4.52	3.50	3.92	4.41	3.71	3.86	3.15	4.21	4.74	4.79	0.78
Leu	23.08	19.65	20.65	20.87	18.40	20.65	14.45	22.13	21.36	22.84	8.73
Tyr	5.96	6.07	5.77	6.21	4.74	5.90	4.21	7.25	5.73	5.95	5.69
Phe	8.18	7.11	7.53	7.86	6.68	7.07	6.70	8.31	7.96	8.58	4.50
His	1.49	1.49	1.33	1.49	1.37	1.58	1.63	2.32	1.50	1.76	2.50
Lys	0.12	0.09	0.09	0.10	0.53	0.32	1.32	0.97	0.11	0.29	0.10
Arg	1.87	1.98	1.72	1.95	1.92	2.17	3.03	3.64	1.67	1.82	2.56
Trp	0.35	0.28	<0.06	0.27	0.53	0.38	0.46	0.69	0.26	0.69	0.49

^a Protein content is percent based on Kjeldahl nitrogen ($N \times 6.25$).

^b Amino acids are expressed as g/16 g of N.

^c Corn flour (CF); corn gluten meal commercially dried (CGM-CD); distillers' grains with solubles freeze-dried (CDGS-1), and commercially dried (CDGS-3).

^d Dithiothreitol.

contents and amino acid compositions of representative isolates. The protein contents are based on $N \times 6.25$, dry basis. It is apparent that only the commercial zein and β -zein were essentially pure protein. The zein isolated from corn gluten meal consisted of 85–87% protein, while the other isolates were 36–69% protein. The low protein contents undoubtedly reflect the presence of xanthophyll pigments and lipids that are normally removed during commercial preparation by extraction with hexane (Swallen 1941) or by fractional precipitation of the zein by cooling (Carter and Reck 1970). The presence of the pigments was obvious from the brownish-yellow to yellow color of the ethanol extracts, but they were not dialyzable and, hence, not removed. The amino acid composition of commercial zein and α -zein were similar as might be expected from their similarity on gel electrophoresis (Figs. 1, 3, and 4). In contrast, β -zein differed considerably from the commercial zein and α -zein. The crude isolates from corn flour and corn gluten meal generally paralleled the compositions of commercial zein and α -zein. The CDGS-1 isolates likewise compared fairly closely to the two zeins except for lower values for Glu, Pro, Ala, Leu, and Phe for the isolate prepared without reducing agent present. The crude zein isolated from CDGS-3 with ethanol likewise was low in Glu, Pro, Ala, Leu, and Phe but high in Lys and Arg. The zein isolated from CDGS-3 with ethanol + DTT was high in Asp, Gly, Cys, Val, Met, Tyr, and Lys. CDGS-3 was prepared from a mixture of corn, rye, and malted barley. Hence, small amounts of ethanol-soluble fractions from the noncorn cereals may also have been extracted and influenced the amino acid analyses.

CONCLUSIONS

Our results show that at least a part of the zein and other corn proteins survive the gelatinization, fermentation, distillation, and drying operations in the production of CDGS, but these proteins are difficult to extract, requiring the use of NaOH, DTT, and SDS. Even under these drastic extraction conditions, we accounted for only about one-third of the total nitrogen, although some of this includes NPN and yeast protein. It is not known whether the non-extractable proteins have been degraded or modified through interactions with carbohydrates. The latter possibility seems most

likely because we saw little evidence of degradation in the extractable portion of the proteins, and the CDGS samples were brown, indicative of Maillard reactions. Crude zein can be isolated from CDGS by extracting with ethanol or ethanol + DTT, but the yields are so low that this does not appear to be an economically viable consideration when one can isolate zeins in yields of $\approx 50\%$ of the weight of corn gluten meal. The true yields of zein from CDGS are even lower than indicated in Table II because of the coextraction of pigments and lipids that resulted in protein purities of only 37–57% (Table III). It remains to be demonstrated whether the properties of the residual zein in CDGS can be utilized by incorporating CDGS directly into products such as biodegradable polymers or structural materials.

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