# Protein-Rich Alcohol Fermentation Residues from Corn Dry-Milled Fractions

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Cereal Chem. 62(6):470–473

ABSTRACT

Grits, flour, degerminator meal, and hominy feed from corn dry-milling were each fermented to produce ethanol. The residues, after ethanol was distilled, were fractionated into distillers' grains, centrifuged solids, and stillage solubles. Corn grits distillers' grains had 68% protein and accounted for 49% of the fermentation residue. Most of the nitrogen in stillage solubles passed through membrane with a 10,000 molecular weight cutoff. Nitrogen compounds in distillers' grains were less soluble than those of the dry-milled fraction from which they were derived. Lysine was considerably enriched in degerminator meal, hominy feed, and their fermentation products compared to that in grits, flour, and their distillers' grains. Fermentation of corn dry-milled fractions to make ethanol results in a number of by-products potentially superior to those of corn distillers' grains.

Ethanol production by fermentation is a growing industry in the United States: nearly 600 million gal were being produced annually as of January 1984 (Hallberg 1984). The predominant feedstock for this process is corn, although small amounts of wheat, sorghum, and barley are also used. Fermentation of cereal grains to make alcohol also produces a protein-rich by-product (stillage) that remains after alcohol is distilled.

Fermentation utilizes primarily starch, so other nutrients, such as protein, are concentrated. Several investigators have characterized and found uses for those by-products. Fractionation and characterization of corn stillage, corn distillers' dried grains with and without solubles, have been reported (Wu et al 1981, Wu and Stringfellow 1982). Satterlee et al (1976) prepared protein concentrate from fermented corn and fermented wheat by extraction with alkali. Tsen et al (1982, 1983) incorporated distillers' dried grain flours in bread and cookies. Finley and Hanamoto (1980) incorporated various fractions of brewers' spent grains into bread. Brewers' spent grain was also blended with flour for muffin and cookie formulations (Prentice 1978, Prentice et al 1978). Sweeten et al (1983) reported fractionation and composition data of corn and sorghum stillage.

Although corn dry-milling optimizes yields of prime goods (low-fat grits, meal, flour, and oil), about one-third of the corn after dry-milling remains as hominy feed, consisting primarily of degerminator meal, corn bran, bran meal, deoiled germ, and broken grain. The level of protien in hominy feed exceeds that in corn, and the lysine and tryptophan contents of the hominy feed protein are superior to that of whole corn or corn grits (Wall et al 1971). Pilot-plant production of ethanol from whole ground corn, corn flour, and starch has been studied (Yang et al 1981). However, fermentations of corn grits, degerminator meal, and hominy feed to make ethanol have not been reported. This paper reports the use of yellow corn grits, flour, degerminator meal, and hominy feed to produce alcohol via fermentation and describes the fractionation and characterization of the stillages resulting from fermentation of these corn dry-milled fractions.

## MATERIALS AND METHODS

### Fermentation

Corn grits, flour, degerminator meal, and hominy feed from dry-milling of corn were from the mill and processing streams of Illinois Cereal Mills, Paris, IL. Each medium for fermentation was prepared in duplicate to approximately 20% glucose equivalent; dry weights used were 1,821, 1,909, 2,069, and 2,568 g of grits, flour, degerminator meal, and hominy feed, respectively, dispersed in 5 L of tap water. Each fermentation was performed in a 20-L stainless steel, temperature-controlled, jacketed fermentor equipped with stirrers. Ammonium sulfate (40 g) was added to corn grits as a nitrogen source for yeast. Alpha-amylase was added to convert starch to soluble dextrins, and glucoamylase was used to hydrolyze dextrins to glucose; yeast then converted glucose to ethanol. Each fermentation was stopped after 66 hr. Details of the fermentation procedures were reported previously (Wall et al 1983).

### Fractionation of Stillage

Alcohol was distilled from the fermentor by steam ("live" for grits and "jacket" for the rest), and the residue (stillage) was collected by suction filtration through cheesecloth. The materials that remained on the cheesecloth were "distillers' grains." The thin stillage that passed through the cheesecloth was centrifuged at 45,000 rpm in a Model T-1 Sharples continuous centrifuge with a 4.5-cm inside diameter bowl. The resulting supernatant was termed "stillage solubles," whereas solids in the centrifuge bowl were designated "centrifuged solids." More details were described elsewhere (Wall et al 1983).

### Approximate Molecular Size of Nitrogen Components of Stillage Solubles

A Model 52 ultrafiltration cell (Amicon Corp., Lexington, MA) equipped with 43-mm diameter membranes with nominal molecular weight cutoffs of 500 (UM 05) and 10,000 (PM 10) was used. For each experiment, stillage solubles (10–20 ml) were pipetted into the cell above the membrane, and distilled water was added under 50 lb/in.$^2$ of nitrogen. A solution equal in volume to approximately four times the volume of the sample passed through the membrane (permeate). Volumes of solution above the membrane (concentrate) and of permeate were measured, and nitrogen and solids contents of each solution were determined.

### Protein Extraction

Corn grits and hominy feed (10 g) were extracted at room temperature sequentially with 100 ml of water, 1% sodium chloride, 70% ethanol, 70% ethanol plus 0.1% dithiothreitol (DTT), and borate plus 0.5% sodium dodecyl sulfate (SDS) and 0.1% DTT at pH 10.7 (Method 1). In addition to Method 1, distillers' grains from grits (1.5 g) and from hominy feed (5 g) were also sequentially extracted with 100 ml of water, 1% sodium chloride, 70% ethanol, 70% ethanol plus 0.1% DTT, 0.1*N* sodium hydroxide plus 0.1% DTT at pH 11.9, and 0.1*N* sodium hydroxide plus 0.5% SDS and 0.1% DTT at pH 11.9 (Method 2). Further details were reported by Wu et al (1981).

### Analyses

Nitrogen, fat, fiber, and ash contents were determined by AACC Approved Methods (1976), and protein was calculated from N $\times$ 6.25. Although a nitrogen to protein conversion factor of 5.9 was

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reported for corn flour (Ewart 1967), this value has not been generally accepted. Moisture was determined by heating samples in an air oven at 100°C to constant weight; solids content (dry matter) was determined by weighing the residue from a known volume of solution dried overnight in an air oven at 100°C and then dried for 3 days in a vacuum oven at 100°C. Starch was determined by a polarimetric method (Garcia and Wolf 1972). High-performance liquid chromatography on a Bio-Rad HPX87H (300 × 7.8 mm) column (Richmond, CA) with 0.01*N* sulfuric acid eluant at 45°C was used to analyze glucose and ethanol (Wall et al 1983). Solids, ash, and moisture determinations were analyzed in duplicate, and nitrogen determinations were performed in quadruplicate.

Samples for amino acid analysis were hydrolyzed for 24 hr by refluxing in 6*N* hydrochloric acid. A portion of the acid hydrolysate, dissolved in pH 2.2 citrate buffer, was analyzed using a Glenco MM-100 amino acid analyzer; data were computed by the method of Cavins and Friedman (1968).

## RESULTS AND DISCUSSION

### Composition and Yield of Fermentation Products

The average concentrations of ethanol after 66 hr of fermentation were 7.8, 7.3, 7.2, and 8.1% by weight for grits, flour, degerminator meal, and hominy feed, respectively. The attained ethanol yields (percentage of theoretical) were 85, 76, 95, and 91% for grits, flour, degerminator meal, and hominy feed, respectively. The lower alcohol yield (76%) for corn flour is consistent with high residual glucose (3.9%) in the final fermentor beer; longer fermentation may be needed for corn flour to achieve more complete utilization of glucose and high yield of ethanol. Yang et al (1981) also reported incomplete utilization of sugar in fermenting corn flour. Ethanol yields from corn grits and hominy feed did not differ greatly from that of corn treated under identical experimental conditions (Wall et al 1983), whereas the yield from degerminator meal was higher than that of corn.

Table I lists compositions and yields of fermentation products from corn dry-milled fractions. Distillers' grains accounted for the largest percentage of each residue except for corn flour, in which stillage solubles was the largest fraction. Because of the smaller particle size of corn flour, thin stillage that passed through the cheesecloth was still high in suspended solids, which easily clogged the centrifuge bowl. Therefore, thin stillage was passed through a 9XX screen (100 mesh) to remove additional solids before centrifugation. Centrifuged solids were the smallest fraction of fermentation residue for all corn dry-milled fractions.

The corn grits distillers' grains had 68% protein and relatively low fat, fiber, and ash contents and could find use as a food protein concentrate. Distillers' grains from cornflour were also rich in protein (40–47%) and contained less fat and fiber than do distillers' grains from corn (Wu et al 1981, Wall et al 1984). The lower fat contents of distillers' grains from grits and flour may give better taste and storage stability, because defatting barley distillers' grains improved their acceptability by taste panel (Dawson et al 1984). Distillers' grains from degerminator meal and hominy feed were similar in protein, fat, and fiber content to corn distillers' grains. Stillage solubles from grits and flour contained more protein but less ash than similar fractions from degerminator meal and hominy feed.

**TABLE I**
**Yield and Composition of Fermentation Products from Corn Dry-Milled Fractions (Dry Basis)**

| Dry-Milled Fractions[a] | Percent of Residue | Content (%) Protein (N × 6.25) | Fat | Fiber | Ash | Starch |
|---|---|---|---|---|---|---|
| Grits | | | | | | |
| (25% residue)[b] | | 11.1 | 1.0 | 0.5 | 0.4 | 84.1 |
| DG | 49 | 67.5 | 2.8 | 3.8 | 0.9 | 8.5 |
| CS | 14 | 33.3 | 0.8 | 4.2 | 1.2 | 9.7 |
| SS | 37 | 30.2 | nd[c] | nd | 7.8 | nd |
| Flour | | | | | | |
| (34% residue) | | 8.8 | 1.9 | 0.3 | 0.5 | 85.8 |
| DG on cheesecloth | 22 | 47.4 | 5.4 | 6.8 | 1.3 | 10.9 |
| DG on 9XX | 22 | 39.7 | 2.8 | 3.6 | 1.8 | 19.2 |
| CS | 3 | 43.4 | 6.9 | 8.7 | 1.3 | 10.8 |
| SS | 53 | 34.1 | nd | nd | 3.3 | nd |
| Degerminator meal | | | | | | |
| (40% residue) | | 13.6 | 5.8 | 5.0 | 2.3 | 60.4 |
| DG | 81 | 28.8 | 13.4 | 14.8 | 2.5 | 9.2 |
| CS | 3 | 43.9 | 4.4 | 8.4 | 3.4 | 15.6 |
| SS | 16 | 14.7 | nd | nd | 21.1 | nd |
| Hominy feed | | | | | | |
| (44% residue) | | 10.5 | 5.2 | 6.0 | 1.5 | 81.1 |
| DG | 89 | 22.3 | 11.0 | 15.6 | 2.5 | 7.3 |
| CS | 1 | 47.6 | 4.5 | 8.9 | 3.2 | nd |
| SS | 10 | 15.4 | nd | nd | 20.1 | nd |

[a] DG = distillers' grains, CS = centrifuged solids, and SS = solubles.
[b] In parentheses, weight of each fermentation residue as a percentage of the dry-milled fraction.
[c] nd = not determined.

### Nitrogen Distribution and Content of Stillage Solubles

The nitrogen distribution and content of stillage solubles from corn dry-milled fractions are shown in Table II. The results with the UM 05 membrane indicated that 35–66% of the nitrogen was above 500 molecular weight. Nitrogen contents of concentrates from the UM 05 membrane were also higher than those of the permeate fractions. Ultrafiltration of the stillage solubles with PM 10 membrane (nominal molecular weight cutoff of 10,000) indicated that only 12–30% of the total nitrogen was above 10,000 molecular weight. Nitrogen contents of concentrate fractions from the PM 10 membrane were higher than those of the permeate fractions (lower molecular weight). In comparison, 52% of the total nitrogen was above 500 molecular weight, whereas all nitrogenous materials from stillage solubles of whole corn were smaller than 10,000 molecular weight (Wu et al 1981).

### Protein Fractions of Corn Grits, Hominy Feed, and Their Distillers' Grains

In Method 1, water, 1% sodium chloride, 70% ethanol, 70% ethanol plus 0.1% DTT, and borate plus 0.5% SDS and 0.1% DTT, pH 10.7, were used sequentially to extract albumin, globulin, prolamin, cross-linked prolamin (alcohol-soluble reduced glutelin) and glutelin, respectively. By Method 1, 16% of grits nitrogen was undissolved, but 47% of the nitrogen from grits distillers' grains remained insoluble (Table III). A large decrease in the 70% ethanol (with and without DTT) extracts was observed for the distillers' grains compared with those of grits, largely explaining the increased residual nitrogen in distillers' grains. Presumably the

**TABLE II**
**Nitrogen Distribution and Content of Stillage Solubles from Corn Dry-Milled Fractions**

| Corn Fraction | Membrane[a] | Fraction | Percent of Total N | N Content (% d.b.) |
|---|---|---|---|---|
| Grits | UM 05 | Concentrate | 35 | 8.53 |
| | | Permeate | 65 | 4.73 |
| | PM 10 | Concentrate | 17 | nd[b] |
| | | Permeate | 83 | 4.67 |
| Flour | UM 05 | Concentrate | 56 | 2.82 |
| | | Permeate | 44 | 0.27 |
| | PM 10 | Concentrate | 30 | 5.03 |
| | | Permeate | 70 | 0.34 |
| Degerminator meal | UM 05 | Concentrate | 66 | 5.04 |
| | | Permeate | 34 | 1.46 |
| | PM 10 | Concentrate | 17 | 4.12 |
| | | Permeate | 83 | 2.26 |
| Hominy feed | UM 05 | Concentrate | 59 | 4.09 |
| | | Permeate | 41 | 1.25 |
| | PM 10 | Concentrate | 12 | 3.51 |
| | | Permeate | 88 | 2.22 |

[a] UM 05 and PM 10 membranes have nominal molecular weight cutoffs of 500 and 10,000, respectively.
[b] nd = not determined.

lower solubility of grits distillers' grains nitrogen than that of grits nitrogen is caused by heat denaturation of proteins. Because about half the nitrogen in grits distillers' grains remained in residue 1 from Method 1, an extraction scheme containing stronger solvents (Method 2) was used. Only 8% of the nitrogen from grits distillers' grains was undissolved by Method 2 (Table III).

Compared with grits, hominy feed contains more albumins and globulins but less prolamins and alcohol-soluble reduced glutelin (Table III). Hominy feed also had more nitrogen left in residue 1 than did grits (Method 1). Hominy feed distillers' grains nitrogen was even less soluble than that in hominy feed and grits distillers' grains by Method 1, because 69% of the nitrogen in hominy feed distillers' grains remained in residue 1. A large decrease in 70% ethanol (with and without DTT) extracts was also observed for hominy feed distillers' grains compared with those of hominy feed; in addition, solubility in other solvents was significantly reduced. After alkali extractions, only 20% of the total nitrogen from hominy feed distillers' grains remained in residue 2 (Method 2). In comparison, 51% of the total nitrogen from corn distillers' grains remained in residue 1 (Method 1) and 18% of the total nitrogen was in residue 2 (Method 2) (Wu et al 1981).

**TABLE III**
**Percent Nitrogen Distribution of Soluble Fractions of Corn Grits, Hominy Feed, and Their Distillers' Grains**

| | Percent of Total N | | | |
|---|---|---|---|---|
| Fraction[a] | Grits | Grits Distillers' Grains | Hominy Feed | Hominy Feed Distillers' Grains |
| Either method | | | | |
| Water extract | 2 | 3 | 10 | 4 |
| 1% Sodium chloride extract | 2 | 1 | 7 | 5 |
| 70% Ethanol extract | 29 | 3 | 13 | 3 |
| 70% Ethanol + 0.1% DTT extract | 17 | 1 | 7 | 1 |
| Method 1 | | | | |
| Borate + 0.5% SDS + 0.1% DTT extract, pH 10.7 | 29 | 37 | 32 | 19 |
| Residue 1 | 16 | 47 | 31 | 69 |
| Method 2 | | | | |
| 0.1*N* Sodium hydroxide + 0.1% DTT extract, pH 11.9 | | 42 | | 32 |
| 0.1*N* Sodium hydroxide + 0.5% SDS + 0.1% DTT extract, pH 11.9 | | 32 | | 32 |
| Residue 2 | | 8 | | 20 |

[a]SDS = sodium dodecyl sulfate, DTT = dithiothreitol.

## Amino Acid Composition

The amino acid compositions of corn grits and their fermentation products are listed in Table IV. Grits has high glutamic acid, proline, and leucine but low lysine contents. Grits and grits distillers' grains had similar amino acid composition. Both centrifuged solids and stillage solubles from grits had higher aspartic acid, threonine, glycine, valine, lysine, and arginine but lower glutamic acid, proline, and leucine than corn grits and the grits distillers' grains.

Table IV also shows the amino acid composition of corn flour and its fermentation products. The amino acid compositions of corn flour, corn flour distillers' grains on cheesecloth, on 9XX, and centrifuged solids were close to those of grits except that corn flour and its centrifuged solids had higher lysine. Corn flour stillage solubles had higher glycine, lysine, histidine, and arginine but lower glutamic acid, alanine, methionine, leucine, tyrosine, and phenylalanine than corn flour and its distillers' grains and centrifuged solids.

Degerminator meal had higher aspartic acid, glycine, valine, lysine, and arginine but lower glutamic acid, proline, leucine, tyrosine, and phenylalanine than corn flour (Table IV). Distillers' grains and centrifuged solids from degerminator meal had amino acid compositions similar to those of degerminator meal. The stillage solubles from degerminator meal had higher glycine, lysine, histidine, and arginine but lower valine, isoleucine, leucine, tyrosine, and phenylalanine than those of degerminator meal and its distillers' grains and centrifuged solids.

Hominy feed and its distillers' grains and centrifuged solids had similar amino acid compositions, and the compositions were close to those of degerminator meal (Table IV). Compared with hominy feed and its distillers' grains and centrifuged solids, hominy feed stillage solubles had higher threonine, glycine, and lysine but lower valine, methionine, isoleucine, leucine, phenylalanine, and tyrosine.

**TABLE IV**
**Amino Acid Composition[a] of Corn Grits, Flour, Degerminator Meal, Hominy Feed, and Their Fermentation Products**

| | | Grits | | | Flour | | | | | | Meal[b] | | | | Feed[c] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | DG[d] on | | | | | | | | | | | |
| Amino Acids | Grits | DG | CS[e] | SS[f] | Flour | Cloth | 9XX | CS | SS | Meal | DG | CS | SS | Feed | DG | CS | SS |
| Aspartic | 6.0 | 5.9 | 8.5 | 10.1 | 6.3 | 6.9 | 6.8 | 7.6 | 7.8 | 7.3 | 7.7 | 8.6 | 7.4 | 7.2 | 7.6 | 7.8 | 7.9 |
| Threonine | 3.5 | 3.5 | 4.8 | 5.2 | 3.7 | 4.0 | 4.0 | 4.4 | 4.3 | 3.9 | 4.2 | 4.5 | 4.5 | 4.3 | 4.2 | 4.4 | 5.1 |
| Serine | 5.3 | 5.3 | 5.3 | 6.4 | 5.3 | 5.3 | 5.7 | 5.6 | 5.3 | 5.0 | 5.5 | 5.4 | 4.8 | 5.0 | 5.3 | 5.4 | 5.1 |
| Glutamic | 22.8 | 22.6 | 16.7 | 13.5 | 22.8 | 21.7 | 21.0 | 19.5 | 15.5 | 18.1 | 16.8 | 15.4 | 15.6 | 16.4 | 17.8 | 15.9 | 15.7 |
| Proline | 12.9 | 10.9 | 7.6 | 6.3 | 10.3 | 10.6 | 10.2 | 9.1 | 9.4 | 9.1 | 7.6 | 6.1 | 6.9 | 9.2 | 8.2 | 8.1 | 7.5 |
| Glycine | 3.1 | 2.8 | 4.2 | 8.5 | 3.7 | 3.7 | 4.0 | 4.2 | 8.3 | 4.8 | 4.8 | 4.9 | 9.0 | 5.1 | 4.9 | 4.8 | 8.5 |
| Alanine | 8.5 | 8.8 | 7.4 | 7.4 | 8.2 | 8.4 | 8.2 | 7.9 | 6.2 | 7.5 | 7.3 | 6.8 | 6.8 | 7.0 | 7.6 | 6.9 | 6.8 |
| Valine | 4.2 | 4.6 | 6.2 | 5.0 | 4.6 | 5.1 | 5.7 | 6.1 | 4.6 | 5.7 | 5.9 | 6.0 | 3.6 | 5.3 | 5.6 | 5.9 | 4.2 |
| Cystine | 0.8 | 1.7 | 1.1 | 0.8 | 0.5 | 1.5 | 1.9 | 1.6 | 1.0 | 1.0 | 1.5 | 1.2 | 1.3 | 1.1 | 1.0 | 1.4 | 1.5 |
| Methionine | 1.8 | 1.7 | 1.6 | 0.4 | 1.9 | 1.7 | 2.4 | 1.8 | 0.5 | 1.6 | 0 | 1.6 | 0.6 | 1.5 | 1.8 | 1.6 | 0.6 |
| Isoleucine | 3.0 | 4.1 | 4.8 | 2.9 | 3.7 | 4.1 | 4.3 | 4.5 | 2.6 | 3.2 | 3.8 | 3.9 | 1.9 | 3.2 | 3.7 | 4.0 | 1.9 |
| Leucine | 15.6 | 15.7 | 11.6 | 5.0 | 14.7 | 15.5 | 12.7 | 12.7 | 5.7 | 11.0 | 11.2 | 10.0 | 3.6 | 9.9 | 11.7 | 10.4 | 3.7 |
| Tyrosine | 5.2 | 5.3 | 4.7 | 2.6 | 4.9 | 5.3 | 5.5 | 5.4 | 2.6 | 4.2 | 4.3 | 4.3 | 2.5 | 3.9 | 4.6 | 4.4 | 2.5 |
| Phenylalanine | 5.7 | 6.2 | 5.7 | 2.8 | 5.7 | 5.8 | 6.2 | 5.2 | 2.5 | 4.7 | 5.2 | 5.0 | 1.6 | 4.8 | 5.3 | 5.5 | 1.6 |
| Lysine | 1.3 | 1.5 | 5.4 | 6.0 | 2.2 | 1.4 | 1.3 | 2.3 | 5.4 | 3.9 | 4.4 | 5.1 | 6.6 | 4.3 | 3.9 | 4.9 | 6.4 |
| Histidine | 2.8 | 2.6 | 2.7 | 2.4 | 2.8 | 2.5 | 2.9 | 2.6 | 4.4 | 2.7 | 2.6 | 2.7 | 3.7 | 3.0 | 2.9 | 2.8 | 3.5 |
| Arginine | 3.5 | 3.6 | 5.0 | 6.3 | 4.0 | 3.2 | 3.8 | 4.4 | 6.2 | 6.4 | 6.8 | 7.2 | 8.7 | 7.4 | 5.5 | 6.5 | 7.5 |

[a]Grams of amino acid per 16 g of nitrogen recovered. Tryptophan not determined.
[b]Meal = degerminator meal.
[c]Feed = hominy feed.
[d]DG = distillers' grains.
[e]CS = centrifuged solids.
[f]SS = stillage solubles.

Corn is deficient in lysine for human consumption (Wu and Sexson 1976). The nutrition values, based on lysine contents, of corn products arranged in increasing order are grits, flour distillers' grains on 9XX, flour distillers' grains on cheesecloth, grits distillers' grains, flour, flour centrifuged solids, corn, degerminator meal, hominy feed distillers' grains, hominy feed, degerminator meal distillers' grains, hominy feed centrifuged solids, degerminator meal centrifuged solids, grits centrifuged solids, flour stillage solubles, grits stillage solubles, hominy feed stillage solubles, and degerminator meal stillage solubles.

## CONCLUSION

Distillers' grains from corn grits and flour have higher protein and lower fat contents than corn distillers' grains (Wu et al 1981). The lower fat content may give better taste and storage stability for distillers' grains from corn grits and flour. The lower fiber contents of distillers' grains from corn grits and flour may be desirable in products such as baby foods, in which fiber content may be a limiting factor. Fermentation products from degerminator meal and hominy feed are relatively rich in lysine, the limiting amino acid in corn. Thus, fermentation of corn dry-milled fractions to make ethanol results in a number of by-products potentially superior to corn distillers' grains in various ways, including higher protein content, better nutritional value, lower fat content, and lower fiber content.

## ACKNOWLEDGMENTS

We thank N. E. Harrison for technical assistance, J. F. Cavins for amino acid analyses, J. E. VanCauwenberge for the yeast culture, and Illinois Cereal Mills for corn dry-milled fractions.

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[Received August 27, 1984. Accepted May 24, 1985.]