Protein-Rich Residue from Corn Alcohol Distillation: Fractionation and Characterization[1]

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ABSTRACT

The material from the base of stills after corn alcohol distillation was screened to separate a slurry from screened residue. The slurry was centrifuged to obtain a centrifuge cake and a supernatant. The supernatant contained materials that were smaller than 10,000 in molecular weight and accounted for 20% of the dry weight and 20% of the total nitrogen. The screened residue and the centrifuge cake were combined into base-of-still residue, which accounted for 80% of the dry weight and 80% of the total nitrogen. The base-of-still residue was extracted sequentially with water, sodium chloride, 70% ethanol, 70% ethanol plus dithiothreitol, sodium hydroxide plus dithiothreitol, and sodium hydroxide plus sodium dodecyl sulfate plus dithiothreitol at pH 11.9. The nitrogen content and amino acid composition of each fraction were determined. The nitrogen in base-of-still residue was considerably less soluble than that in ground corn when extracted with water, sodium chloride, 70% ethanol, 70% ethanol plus dithiothreitol, and borate plus sodium dodecyl sulfate plus dithiothreitol at pH 10. The lower nitrogen solubility of the base-of-still residue is probably a result of denaturation of protein and may account for the greater feed efficiency for ruminants of corn distillers' grains than of corn. The denatured protein is degraded less in the rumen, and a higher proportion is digested and absorbed from the lower gastrointestinal tract for maximum growth.

Fermentation of cereal grains to make alcohol produces a protein-rich material (spent grain stillage) after alcohol is distilled. The fermentation process predominantly uses the starch in cereal grains, and other nutrients such as protein are concentrated threefold. Most of the spent grain stillage is recovered in one of three forms as a dry feed ingredient. Distillers' dried grains with solubles (CDDGS) is dried base-of-still material (whole stillage). When this is screened, the part that remains on top (on-screen residue) is pressed and dried; it is then known as distillers' dried grains. The part passing through the screen (off-sceen material) is concentrated in an evaporator and drum dried; it is called distillers' dried solubles. Obtaining the maximum economic return from stillage, which has 5–10% solids, with a minimum energy input is a significant part of the fuel alcohol program for farmers and industrial companies.

Satterlee et al (1976) studied the chemical, functional, and nutritional characterization of protein concentrates from distillers' grains by extraction with alkali. Feeding studies utilizing distillers' dried grains with solubles or distillers' dried solubles have been conducted on beef cattle (Chen et al 1977, Hatch el at 1972), dairy cattle (Loosli et al 1961, Warner et al 1957), calves (Fries et al 1956, Schabinger and Knodt 1948), sheep (El Hag 1969), swine (Thong et al 1978, Wahlstrom et al 1970), chicken (Harms et al 1969, Matterson et al 1966, Scott et al 1955), and turkey (Atkinson et al 1955). Little published information is available on fractionation of stillage, however. This article reports fractionation and characterization of wet, protein-rich residue from corn alcohol distillation, emphasizing the composition of different fractions and on protein classes separated by various solvents.

MATERIALS AND METHODS

CDDGS and base-of-still, on-screen, and off-screen materials were from a local distillery. The grain was 99% corn and 1% barley malt. Corn had been ground to 10–20 mesh. The amount of yeast used was 2.25 million yeast cells per milliliter of mash or about 0.05% of the corn weight. CDDGS, supplied in dry form, was ground twice in a hammer mill equipped with a screen containing holes of 1/16 in diameter. All other materials were supplied in wet form while hot and were stored at 4° C. Base-of-still material had been screened into on-screen and off-screen fractions at the distillery.

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[2]The mention of firm names or trade products does not imply endorsement or recommendation by the USDA over other firms or similar products not mentioned.

Fractionation of Base-of-Still Material and Off-Screen Material

The base-of-still material was fractionated (Fig. 1) by a 20-mesh screen into an on-screen residue and a slurry. The slurry (off-screen material) was centrifuged at 10,400 × *g* for 10 min to obtain a centrifuge cake (off-screen residue) and a supernatant. The on-screen and off-screen residues were combined and freeze-dried to obtain base-of-still residue. The off-screen supernatant was freeze-dried to give base-of-still supernatant. When the base-of-still material was centrifuged directly, poor separation of solid and liquid resulted at 3,300 and 10,400 × *g*. However, when most solids were removed first by screening, good separation of solid and liquid was obtained by centrifuging the slurry that passed through the screen.

Protein Extraction From Base-of-Still Residue

Base-of-still residue (5 g) was put in a stainless steel cup with 100 ml of solvent and blended for 5 min in a Waring Blendor (Fig. 2). The sample after blending was centrifuged at 10,400 × *g* for 10 min. For Method 1, the solvents used sequentially were water, 1% NaCl, 70% ethanol, 70% ethanol plus dithiothreitol (DTT), and borate (0.125 *M* borax plus 0.043 *N* sodium hydroxide plus 0.425 *M* sodium chloride) plus 0.5% sodium dodecyl sulfate plus 0.1% DTT at pH 10, as described by Landry and Moureaux (1970). These solvents extract albumin, globulin, prolamin, crosslinked prolamin or alcohol-soluble reduced glutelin, and glutelin, respectively. For

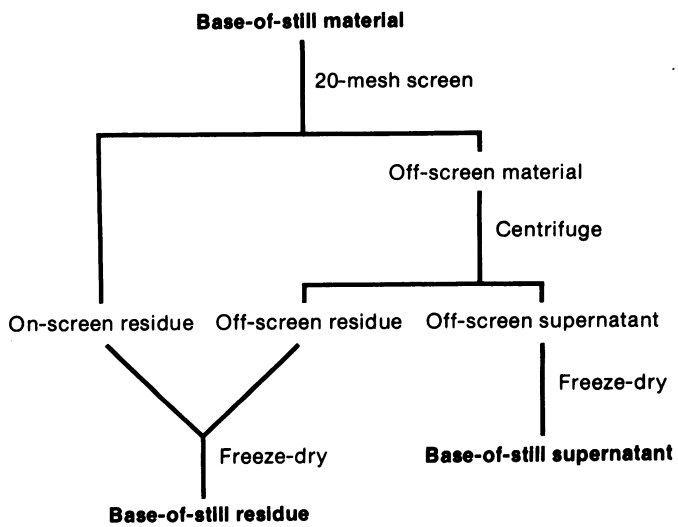

Fig. 1. Separation of base-of-still material.

Method 2, water, 1% NaCl, 70% ethanol, 70% ethanol plus DTT, 0.1*N* sodium hydroxide plus 0.1% DTT at pH 11.9, and 0.1*N* sodium hydroxide plus 0.5% sodium dodecyl sulfate plus 0.1% DTT at pH 11.9 were used sequentially.

Ultrafiltration

Two kinds of ultrafiltration apparatus with different membranes were used. A Millipore immersible CX molecular separator (Millipore Corp., Bedord, MA), consisting of a Pellicon molecular filtration membrane cast on a cylindrical porous plastic core, was immersed in 20 ml of off-screen supernatant in a small vial. When vacuum was applied to the CX separator, solution was sucked through the membrane and collected. A small amount of solution did not go through the separator. This membrane has a nominal molecular weight limit of 10,000; above this level most species are efficiently retained by the membrane. Nitrogen contents of above-CX membrane and through-CX membrane fractions were determined, and the percentage of original nitrogen in each fraction was calculated.

Amicon ultrafiltration cell model 52 (Amicon Corp., Lexington, MA) with two membranes 43 mm in diameter was also used. Each membrance is characterized by its nominal molecular weight cut-off (10,000 for PM10 and 500 for UM05). With the PM10 membrane and 20 ml of off-screen supernatant in the Amicon cell, 20 ml of solution was collected above the membrane and 143 ml below the membrane by feeding in distilled water under nitrogen pressure (50 lb/in.^{2}). For the UM05 membrane and 30 ml of off-screen supernatant in the Amicon cell, 20 ml of solution was collected above the membrane and 40 ml below. Nitrogen contents of each fraction were determined, and the percentage of original nitrogen in each fraction was calculated.

Composition

Protein, lipid, and ash were determined by AACC approved methods (1976). Protein was calculated from N × 6.25 and included any free amino acids and other nitrogen compounds. Phosphorus was determined by a colorimetric procedure (Fiske and Subbarow 1925). Moisture was determined by heating samples to constant weight at 105° C. All determinations were in duplicate except that of protein, for which three to six values were averaged. The number of yeast cells per unit volume was counted by an AO Spencer Bright-Line hemacytometer (American Optical, Buffalo, NY). The percentage of yeast by weight was calculated by counting a suspension of Red Star Distillers' Dry Yeast (Universal Foods Corp., Milwaukee, WI).

For amino acid analysis, each protein sample was hydrolyzed for 24 hr by refluxing in 6*N* hydrochloric acid. The hydrolyzed sample was evaporated to dryness in a rotoevaporator, and the residue was then dissolved in pH 2.2 citrate buffer. A portion of the acid hydrolysate was used in a Beckman Spinco model 121 amino acid analyzer, and the data were computed automatically by the method of Cavins and Friedman (1968). For free amino acid analysis, the sample without hydrolysis was used.

RESULTS AND DISCUSSION

Composition of Corn Distillers' Grains and Solubles Fractions

Protein, lipid, ash, and phosphorus contents of distillers' grains and solubles fractions are listed in Table I. Protein content ranges from 24% for on-screen residue to 42% for off-screen residue. The higher protein content for off-screen residue is a result of yeast cells that have around 50% protein. Base-of-still supernatant and off-screen supernatant are practically identical in composition, which is to be expected from the way they were prepared. The supernatants have low lipid but high ash and phosphorus contents compared with other fractions. The phosphorus content follows the ash content for all fractions. The relatively rich phosphorus content of corn distiller's grains and solubles fractions is an advantage in feeds because phosphorus which is expensive, is essential in animal feeds. The numbers of yeast cell per gram of base-of-still supernatant, base-of-still residue, and off-screen residue were 57,800, 1.61 billion, and 2.68 billion, respectively, on dry basis, or 0.0003, 9.1, and 15.1% by weight, respectively.

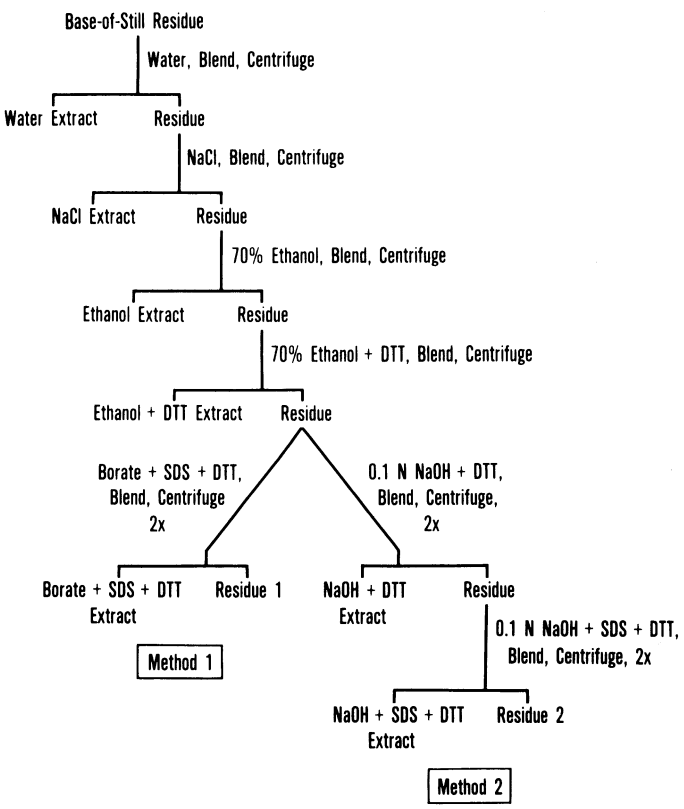

Fig. 2. Fractionation of base-of-still residue by solubility. Certain residues were extracted twice (**2x**) with a particular solvent and the combined extracts used.

TABLE I
Composition of Corn Distiller's Grains and Solubles Fractions (% db)

	Protein (N × 6.25)	Lipid	Ash	Phosphorus	Percent of Total Base-of-Still: Protein	Percent of Total Base-of-Still: Weight
CDDGS[a]	34.1	12.7	5.9	1.2	...	...
Base-of-still						
Supernatant	30.6	1.0	17.8	3.3	20	20
Residue	32.0	16.6	2.9	0.7	80	80
Off-screen						
Supernatant	31.8	1.2	18.2	3.3	20	20
Residue	42.3	17.3	2.7	0.9	35	27
On-Screen residue	23.6	16.5	1.8	0.4	45	53

[a]Corn distillers' dried grains with solubles.

TABLE II
Nitrogen in Off-Screen Supernatant Fractions

	Percent of Original N	N Content (% db)
Inside dialysis bag	0	...
Above PM10 membrane[a]	0	...
Precipitation in boiling 10% trichloroacetic acid	0	...
CX membrane[a]		
Above	6	2.34
Through	94	5.64
UM05 membrane[a]		
Above	52	6.58
Through (4 volumes)	48	6.30
Free amino acids	28	...

[a]The PM10, CX, and UM05 membranes have nominal molecular weight cut-offs of 10,000, 10,000, and 500, respectively.

Although the yeast cell count is relatively accurate, the percentage of yeast by weight is less accurate. The high protein content of all fractions is the major basis for the use of distillers grains as feed and for their possible use in foods.

Table I also shows the protein and weight distributions of different fractions as a percent of total base-of-still material. The percentage figures were rounded off to the nearest integer. Base-of-still supernatant or off-screen supernatant accounts for 20% of the protein and 20% of the weight of base-of-still material. Off-screen residue, however, accounts for 35% of the protein and only 27% of the weight of base-of-still material. Using the off-screen residue separately may be desirable because of its higher protein content.

Off-Screen Supernatant Properties

Table II shows some of the properties of off-screen supernatant. When dialyzed against distilled water, off-screen supernatant retained no nitrogen in the bag. Although a slight precipitate was observed when the supernatant was made to 10% trichloroacetic acid and boiled, no nitrogen was precipitated. No nitrogen was held back by the PM10 membrane and only 6% of the nitrogen was retained by the CX membrane. These results indicate that off-screen supernatant consists only of molecules of less than 10,000 mol wt. The nitrogen content of CX membrane fractions suggests that the relatively large molecules retained by the membrane are low in protein content. The results with the UM05 membrane indicate that about 50% of the nitrogen is represented by molecules of approximately 500 mol wt or less. The nitrogen contents of the UM05-membrane fractions below and above the membrane are not significantly different. Free amino acids account for 28% of the original off-screen supernatant nitrogen, ie, about 60% of the nitrogen that passed through the UM05 membrane is from free amino acids. Free amino acids account for 5.6% of the total nitrogen of base-of-still material; this amount is much higher than the 1.1% of total nitrogen found for corn (Christianson et al 1965).

TABLE III
Base-of-Still Residue Protein Fractions

Fraction[a]	Protein Content[b] (% as-is)	Percent of Total Protein
From either method		
Base-of-still residue	29.6	
Water extract	22.9	8
NaCl extract	3.1	2
70% Ethanol extract	13.2	2
70% Ethanol plus DTT extract	8.3	2
From Method 1		
Borate plus SDS plus DTT extract, pH 10	6.4	30
Residue 1	21.0	51
From Method 2		
NaOH plus DTT extract, pH 11.9	22.6	28
NaOH plus SDS plus DTT extract	14.6	26
Residue 2	13.1	18

[a]SDS = sodium dodecyl sulfate, DTT = dithiothreitol.
[b]N × 6.25.

Base-of-Still Residue Protein Fractions

Two extraction procedures were used to fractionate protein. The Method 1 series of solvents extracted about 95% of the total corn protein (Landry and Moureaux 1970). However, Table III shows that 51% of the total protein is left in residue 1 when Method 1 is used. The most striking difference between corn protein and base-of-still residue is the almost complete absence of zein (70% ethanol extract) in base-of-still residue, 2% compared with about 40% in corn protein.

The lower protein solubility of base-of-still residue compared with that of corn protein is caused by heat denaturation during distillation of alcohol after fermentation. In ruminants, microorganisms in the rumen degrade part of the protein in feed to ammonia, which would be provided by more economical nitrogen sources, such as urea. The protein that escapes degradation (bypass protein) in the rumen and is digested and absorbed from the lower gastrointestinal tract is essential to achievement of maximum growth for the young ruminant. Klopfenstein et al (1978) found that protein from corn distillers' dried grains and CDDGS was consistently utilized more efficiently by calves and lambs than was soybean meal protein. Apparently, the lower solubility for protein (Table III) correlates with more bypass protein and increased feed efficiency for ruminants.

Because half of the protein from base-of-still residue was not extracted by Method 1 (Table III), Method 2 was developed. Eighteen percent of the total protein remains in residue 2, which is considerably lower than the 51% in residue 1.

Amino Acid Composition

The amino acid compositions of CDDGS and stillage fractions are listed in Table IV. Because CDDGS is derived mostly from corn, with only a small contribution from yeast, the amino acid

TABLE IV
Amino Acid Composition[a] of Corn Distillers' Grains and Soluble Fractions

Amino Acid	CDDGS[b,c]	Base of Still		On-Screen Residue[c]	Off-Screen Residue	Standard Error[d]
		Supernatant[c]	Residue[c]			
Aspartic	6.4	7.9	6.4	6.6	6.7	0.34
Threonine	4.0	5.1	4.1	4.1	3.7	0.11
Serine	5.3	5.5	5.5	5.3	4.9	0.15
Glutamic	20.1	22.5	20.7	19.1	18.8	0.61
Proline	10.2	15.4	9.8	9.2	8.9	0.23
Glycine	4.3	6.9	4.0	4.1	3.5	0.10
Alanine	8.0	8.4	8.3	8.1	8.1	0.19
Valine	5.4	5.8	5.3	5.6	5.1	0.12
Cystine	0.9	1.5	1.5	2.0	1.5	0.71
Methionine	2.5	1.6	2.1	2.1	2.1	0.34
Isoleucine	5.3	4.3	4.2	4.1	4.2	0.38
Leucine	15.1	8.6	14.2	13.5	13.6	0.79
Tyrosine	5.5	2.5	4.7	4.3	4.7	0.52
Phenylalanine	5.2	4.4	6.2	5.6	6.2	0.26
Lysine	3.1	3.4	3.1	3.2	2.5	0.14
Histidine	2.6	2.8	2.7	3.0	2.4	0.19
Arginine	4.2	3.1	5.2	5.0	4.3	0.16

[a]Grams of amino acid per 16 g of nitrogen recovered.
[b]Corn distillers' dried grains with solubles.
[c]Duplicate runs.
[d]Calculated from the six duplicate runs shown in Tables IV and V.

TABLE V
Amino Acid Composition[a] of Base-of-Still Residue Fractions

Amino Acid	Extract						Extract			
	Water	NaCl	Ethanol	Ethanol + DTT[b]	Borate + SDS[b] + DTT[c]	Residue 1[c]	NaOH + DTT	NaOH + SDS + DTT	Residue 2	Standard Error[d]
Aspartic	8.6	6.3	6.1	5.0	6.8	6.1	6.2	7.8	6.7	0.34
Threonine	4.4	3.9	3.2	4.2	4.0	3.6	2.1	2.9	3.9	0.11
Serine	5.0	4.4	4.8	5.6	4.9	5.2	2.5	3.7	5.1	0.15
Glutamic	17.8	16.1	22.7	23.6	17.4	19.5	15.1	24.7	19.0	0.61
Proline	12.7	10.4	9.0	11.4	8.0	8.6	7.7	10.7	8.5	0.23
Glycine	6.2	5.4	2.6	2.9	4.6	3.1	3.7	3.2	3.2	0.10
Alanine	7.5	5.3	8.7	8.9	6.7	8.3	6.4	10.8	8.6	0.19
Valine	5.3	4.7	4.3	4.1	5.9	4.9	5.4	5.7	5.5	0.12
Cystine	1.6	1.9	0.8	0.1	2.7	1.5	1.9	2.0	2.2	0.71
Methionine	2.5	1.4	1.4	5.4	2.9	1.7	2.5	2.6	1.6	0.34
Isoleucine	4.0	3.0	3.9	4.0	3.9	4.0	3.2	4.9	4.2	0.38
Leucine	7.4	6.8	16.9	17.3	11.1	14.6	9.5	20.4	14.0	0.79
Tyrosine	1.9	2.5	4.3	5.4	4.7	4.3	4.2	5.9	3.8	0.52
Phenylalanine	3.8	3.0	6.8	6.2	5.1	5.8	4.1	7.5	5.2	0.26
Lysine	3.9	4.3	0.9	0.4	3.6	2.4	6.6	2.3	2.7	0.14
Histidine	2.8	4.2	1.5	1.7	3.0	2.4	3.3	0.9	1.9	0.19
Arginine	2.6	4.4	2.1	1.6	6.3	4.0	8.3	4.0	3.7	0.16

[a] Grams of amino acid per 16 g of nitrogen recovered.
[b] DTT = dithiothreitol, SDS = sodium dodecyl sulfate.
[c] Duplicate runs.
[d] Calculated from the six duplicate runs shown in Tables IV and V.

composition of CDDGS is similar to that of corn (Wu and Sexson 1976). Base-of-still residue has higher phenylalanine and arginine but lower isoleucine than does CDDGS. Because base-of-still residue contributes 80% of the weight and 80% of total nitrogen to CDDGS, their amino acid compositions were not expected to be grossly different. The base-of-still supernatant has more aspartic, threonine, glutamic acid, proline, glycine, and valine but less leucine, tyrosine, phenylalanine, and arginine than does the base-of-still residue. On-screen residue is the predominant fraction of base-of-still residue, and they have a similar amino acid composition, but the difference in glutamic acid and proline may be larger than experimental error.

When the essential amino acid compositions of CDDGS and the stillage fractions are compared with the amino acid pattern for high-quality protein for human consumption (NAS 1980), they meet or exceed all requirements except that for lysine. Tryptophan was not determined.

Table V shows the amino acid compositions of base-of-still residue fractions according to solubility in various solvents. Ethanol and ethanol plus DTT extracts have very low lysine values compared with those of the other fractions. Water and NaCl extracts are relatively rich in lysine, but NaOH plus DTT extract has the highest lysine value by far. Water and NaCl extracts are low in leucine and tyrosine but high in glycine compared with other fractions. Ethanol plus DTT extract has the lowest cystine but the highest methionine content of all fractions. Residues 1 and 2 have the same amino acid composition, except for threonine and maybe histidine and phenylalanine. NaOH plus DTT extract is low in threonine, serine, glutamic acid, and proline but high in arginine compared with the other fractions.

CONCLUSION

The amount and relatively low molecular weight of the base-of-still supernatant indicates that proteolysis of protein occurs during the production of alcohol from corn; corn usually has less low molecular weight nitrogen than was found in the supernatant. The high protein content of off-screen residue, derived from yeast protein, may make this fraction a potential food source, but the nucleic acid content (from yeast) may have to be reduced. Keeping off-screen residue separately may be desirable. The poor solubility of the proteins in distillers' grains compared to that in corn proteins may be the result of denaturation during alcohol distillation. This decreased solubility may be a deficiency in feed for nonruminants, but it appears to be an asset for ruminants because it reduces protein degradation in the rumen and permits better digestion and absorption from the lower gastrointestinal tract.

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