Determination of Neutral Sugars in Corn Distillers' Dried Grains, Corn Distillers' Dried Solubles, and Corn Distillers' Dried Grains with Solubles

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Corn distillers' dried grains (DDG), corn distillers' dried solubles (DDS), and corn distillers' dried grains with solubles (DDGS) are coproducts from ethanol production. Knowledge of the carbohydrate composition of these coproducts will increase their potential for further processing. Materials were first hydrolyzed by trifluoroacetic acid, and the resulting neutral sugars were analyzed by high-performance liquid chromatography. Glycerol (3.5–10.2%), arabinose (2.8–10.0%), xylose (3.2–14.1%), mannose (0–2.6%), glucose (5.6–18.8%), and galactose (1.1–2.6%) were found in these ethanol coproducts. Corn DDS had the highest content of neutral sugars (39%), of which glucose accounted for about half of this total. Neutral carbohydrates from the hydrolysis of the 80% methanol soluble fraction (monosaccharide and low molecular weight oligosaccharides) from these corn distillers' products were mostly glycerol and glucose.

INTRODUCTION

In the United States corn is the most common biomass for commercial ethanol production by fermentation (Morris, 1983). A protein-rich residue (stillage) remains after ethanol is distilled. The stillage is centrifuged or screened to yield an insoluble solid fraction, corn distillers' grains (DDG), and a soluble fraction, corn distillers' solubles (DDS). The soluble fraction is usually evaporated to a syrup and dried with the insoluble solid fraction to produce corn distillers' dried grains with solubles (DDGS).

Corn DDGS is the usual coproduct marketed from ethanol producers. Commercial feasibility of fuel ethanol production depends heavily on the value credited to coproducts. Traditionally, corn DDGS has been used for animal feed.

A number of investigators have incorporated corn DDG with or without solubles into food. Tsen et al. (1982, 1983) used DDG flour to replace part of wheat flour for making cookies and breads. Corn DDG was incorporated in blended food products (corn–soy–milk) (Wall et al., 1984; Bookwalter et al., 1984). O'Palka (1987) and O'Palka et al. (1989) incorporated corn DDGS in breads and baked products. Reddy et al. (1986a,b) evaluated DDG flour as an ingredient in canned meat-based foods and in wheat muffins. Wu et al. (1987) prepared spaghetti supplemented with corn DDG. Wampler and Gould (1984) utilized distillers' spent grain for the manufacture of puff-extruded type products. Kim et al. (1989) extruded DDG blended with cereal flours. Rasco and McBurney (1989) produced human food product from dried distillers' spent cereal grains and solubles.

Although protein, fat, ash, crude fiber, and total dietary fiber contents of corn DDG, corn DDS, and corn DDGS have been reported, little is known concerning the carbohydrate fraction of corn DDG, corn DDS, and corn DDGS. Albersheim et al. (1967) used trifluoroacetic acid for hydrolyzing plant cell-wall polysaccharides, because the yields of most monosaccharides from plant cell-wall preparations are at least equal to those obtained by hydrolysis with mineral acids, and trifluoroacetic acid may be readily removed by evaporation. Albersheim et al. (1967) found 2 N trifluoroacetic acid at 121 °C for 1 h was optimal hydrolysis time for most of the sugars. Dowd et al. (1993) used gas chromatography-mass spectrometry to determine the low molecular weight organics in the soluble part of corn stillage without hydrolysis. This paper reports the neutral sugars in corn DDG, corn DDS, and corn DDGS after trifluoroacetic acid hydrolysis.

MATERIALS AND METHODS

Materials. Corn DDG (Fiber Plus N-6000), corn DDS (Nutrateen S-8000), and corn DDGS (mixed grains MG 102) were from Nutritional Products Co. (Louisville, KY). For simplicity, "corn" is dropped from subsequent mention of DDS, DDG, and DDGS. At the production plant, whole stillage was separated into a solid fraction and thin stillage. Thin stillage was evaporated and dehydrated to yield DDS and stored in a separate silo. The solid fraction was dried to give DDG and stored in another silo. A constant ratio of DDS and DDG was metered and combined to yield DDGS. DDG, DDS, and DDGS were each ground three times in an Alpine Model 160 Z pin mill at 14 000 rpm to reduce particle size for uniform sampling. The materials were stored in 1 °C.

Hydrolysis. Inositol (5 mg) as internal standard was added to 20–60 mg of DDG, DDS, or DDGS before 2 N trifluoroacetic acid (2 mL) hydrolysis for gas chromatography analysis. Hydrolysis was carried out in a 13 × 100 mm tube with a Teflon-lined screw cap for 1 h at 120 °C in an autoclave. After hydrolysis, trifluoroacetic acid was removed completely with Bio-Rad (Richmond, CA) AG 1-X8 100–200-mesh acetate form anion-exchange resin in a Pasteur pipet. The solution was evaporated to dryness in a Speed Vac concentrator (Savant, Framingham, NY).

Gas Chromatography (GC). The dried sample after hydrolysis was treated with 1 mL of pyridine with 45 mg of hydroxyamine hydrochloride and allowed to stand 20 min at 80 °C. Acetic anhydride (1 mL) was added. After 20 min, the content was transferred to a test tube with 1 mL of chloroform and 2 mL of water (Seymour et al., 1975). The material was mixed in a vortex (Scientific Products, McGaw Park, IL) for 30 s. The chloroform layer was transferred to another tube, and washing with water was repeated two times. The chloroform layer was removed and dried with Davison molecular sieves 3Å (Fisher Scientific, Pittsburgh, PA). The sample (1 μL usually) was injected into a Perkin-Elmer Sigma 3B capillary chromatograph (Norwalk, CT) equipped with a Hewlett-Packard (Palo

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Table 1. Neutral Sugar Contents (Percent of Dry Weights) of Total Hydrolysate of Corn Distillers' Products by HPLC

sample	hydrolysate	glycerol	arabinose	xylose	mannose	glucose	galactose	total
corn distillers' solubles	1	10.71 (0.64)[a]	2.89 (0.74)	3.30 (0.16)	2.41 (0.09)	19.80[a]	1.12 (0.25)	40.23
	2	9.70 (0.45)[a]	2.76 (0.52)	3.01 (0.15)	2.74 (0.17)	17.88[a]	1.12 (0.10)	37.21
	mean	10.2 (0.5)	2.8 (0.6)	3.2 (0.2)	2.6 (0.1)	18.8	1.1 (0.2)	38.7
corn disillters' grains with solubles	1	7.58 (0.13)	6.18 (0.85)	8.30 (0.22)[a]	1.72 (0.37)	11.89	1.85 (0.13)	37.52
	2	7.93 (0.25)	6.55 (1.15)	8.67 (0.29)[a]	1.43 (0.54)	11.61	1.96 (0.18)	38.15
	mean	7.8 (0.2)	6.4 (1.0)	8.5 (0.3)	1.6 (0.5)	11.8	1.9 (0.2)	38.0
corn distillers' grains	1	3.82 (0.30)	9.87 (0.40)	14.06	0 (0)	5.64 (0.15)	2.61 (0.15)	36.00
	2	3.22 (0.41)	10.09 (0.43)	14.23	0 (0)	5.58 (0.01)	2.53 (0.08)	35.65
	mean	3.5 (0.4)	10.0 (0.4)	14.1	0 (0)	5.6 (0.1)	2.6 (0.1)	35.8

[a] Difference between duplicate hydrolysates ($p < 0.05$). Value in parentheses is standard deviation from duplicate or triplicate analyses.

Alto, CA) cross-linked methyl silicone ultra 1 capillary column (film thickness, 0.11 μm; internal diameter, 0.20 mm; 25 m long). Injection port and detector temperatures were 275 °C, and carrier gas was helium. The column was held at 110 °C for 3 min, and the temperature was then increased at 5 °C/min to 185 °C. Quantitation was by peak area using inositol as internal standard.

Mass Spectrometry (MS). A Hewlett-Packard 5970 series mass selective detector was used. Sample preparation was the same as described under GC and Hydrolysis.

High-Performance Liquid Chromatography (HPLC). A Waters Model 710B liquid chromatograph (Milford, MA) equipped with an R401 differential refractometer together with an Aminex HPX-87P carbohydrate analysis column (Bio-Rad Laboratories), 300 × 7.8 mm, at 85 °C with water as mobile phase and a flow rate of 0.6 mL/min was used. The sample was hydrolyzed as described under Hydrolysis except no inositol standard was added. After hydrolysis and removal of trifluoroacetic acid, the solution was evaporated to dryness, dissolved in 2 mL of water, and filtered through a 0.45-μm filter. A number of known concentrations of glycerol, arabinose, xylose, mannose, glucose, and galactose were injected into the column as standards. Quantitation was by area, and the sample area was close to the standard area to minimize uncertainty.

Monosaccharides and Low Molecular Weight (LMW) Oligosaccharides. DDG, DDS, and DDGS were each extracted with 80% methanol to remove monosaccharides and LMW oligosaccharides. The 80% methanol extracts were then hydrolyzed for subsequent GC, MS, and HPLC measurements.

Analyses. The moisture content of each solid was determined by heating at 135 °C for 2 h in an air oven (AACC, 1983).

Statistical Analysis. General linear models procedure was used to compare the results from duplicate hydrolysis.

RESULTS AND DISCUSSION

Common to all methods of determining the chemical composition of polysaccharide is an initial acid hydrolysis into constituent monosaccharides, all of which are, to some extent, degraded by acid (Dutton, 1973). The monosaccharides decomposed at a lower rate while they were in the polysaccharide, as opposed to being in the free monosaccharide form (Biermann, 1988). For this reason, a correction factor for hydrolysis losses, under any conditions, can never be completely accurate, and it is preferable to cleave glycosidic linkages with the minimum of decomposition (Biermann, 1988). Albersheim et al. (1967) found 2 N trifluoroacetic acid at 121 °C for 1 h was optimal hydrolysis time for most of the sugars from plant cell-wall preparations, and this method was used in this study.

Preliminary experiments indicated that HPLC gave better results than GC in terms of reproducibility between replicate determinations of the same hydrolysate as well as between different hydrolysates (not shown). MS was used to confirm the identity of GC and HPLC peaks from the characteristics of abundance vs mass/charge pattern by comparison with the known MS pattern of glycerol, arabinose, xylose, mannose, glucose, and galactose. The glycerol peak emerged soon after the solvent front in GC, and the necessity of making volatile derivative may contribute to more uncertainty in GC compared with HPLC.

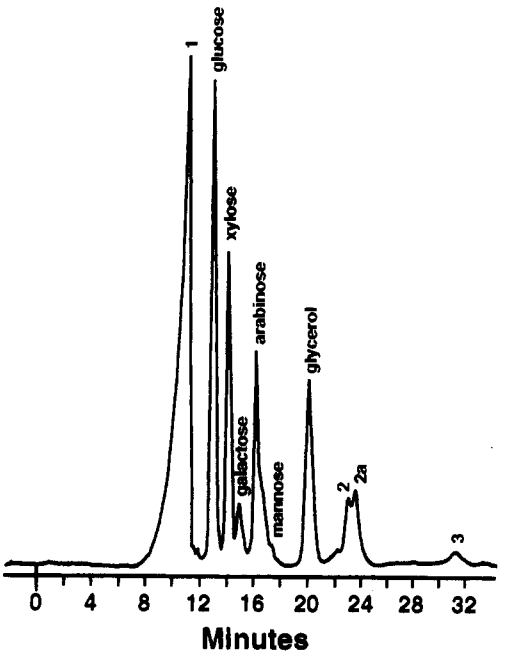

Figure 1. High-performance liquid chromatography pattern of total hydrolysate of corn distillers' dried grains with solubles. Peaks 1, 2, 2A, and 3 are unknown (not carbohydrate).

Figure 1 is the HPLC pattern of total hydrolysate of DDGS. In addition to glucose, xylose, galactose, arabinose, mannose, and glycerol peaks, there are unknown peaks 1, 2, 2A, and 3. The unknown peaks were collected, and negative tests with phenol–sulfuric acid for each unknown peak indicate they are not carbohydrates. When the unknown peaks were each dried and then dissolved in methanol, no peak showed in MS, perhaps because the unknown peaks were not volatile. Trimethylsilyl derivatives of the unknown peaks also did not show any peak by MS. The HPLC patterns of total hydrolysate of DDG and of DDS are qualitatively similar to Figure 1. Also, the 80% methanol soluble fraction of each corn distillers' product is qualitatively similar to Figure 1.

Corn Distillers' Dried Solubles. The largest percentage of neutral sugar from total hydrolysate of DDS is glucose followed by glycerol (Table 1). There is no significant difference ($p > 0.05$) between duplicate hydrolysates of this distillers' product as far as arabinose, xylose, mannose, and galactose are concerned, but there is significant difference ($p < 0.05$) for glycerol and glucose values between duplicate hydrolysates. Single values for glucose resulted from duplicate values that went off scale.

The hydrolysate of monosaccharides and LMW oligosaccharides of DDS were dominated by glycerol and glucose (Table 2). There is no significant difference ($p >$

Table 2. Neutral Sugar Contents (Percent of Dry Weights) of 80% Methanol Soluble Fraction Hydrolysates of Corn Distillers' Products by HPLC

sample	hydrolysate	glycerol	arabinose	xylose	mannose	glucose	galactose	total
corn distillers' solubles	1	9.98 (0.01)	0.85 (0.32)	0.75 (0.18)	0 (0)	11.05 (0.33)	0.36 (0.22)	22.99
	2	10.68 (0.52)	0.93 (0.11)	0.72 (0.11)	0 (0)	11.03 (0.53)	0.35 (0.01)	23.71
	mean	10.3 (0.3)	0.9 (0.2)	0.7 (0.1)	0 (0)	11.0 (0.4)	0.4 (0.1)	23.3
corn distillers' grains with solubles	1	6.28 (0.21)[a]	0.54 (0.12)	0.32 (0.03)	0 (0)	5.25 (0.12)	0.16 (0.01)	12.55
	2	7.13 (0.39)[a]	0.45 (0.27)	0.45 (0.04)	0.07 (0.09)	5.82 (0.37)	0.27 (0.12)	14.19
	mean	6.7 (0.3)	0.5 (0.2)	0.4 (0.0)	0 (0)	5.5 (0.2)	0.2 (0.1)	13.3
corn distillers' grains	1	3.52 (0.35)[a]	0.42 (0.08)	0.29 (0.03)	0.12 (0.05)	1.64 (0.18)	0.14 (0.01)	6.13
	2	2.38 (0.03)[a]	0.48 (0.04)	0.25 (0.11)	0.09 (0.04)	1.59 (0.18)	0.22 (0.04)	5.01
	mean	3.0 (0.2)	0.5 (0.1)	0.3 (0.1)	0.1 (0.0)	1.6 (0.2)	0.2 (0.0)	5.7

[a] Difference between duplicate hydrolysates ($p < 0.05$). Value in parentheses is standard deviation from duplicate analyses.

0.05) between duplicate hydrolysates of this distillers' product for glycerol, arabinose, xylose, mannose, glucose, and galactose. Comparison of Tables 1 and 2 indicates all of the glycerol and most of the glucose of this distillers' product are accounted for by monosaccharides and LMW oligosaccharides.

Corn Distillers' Dried Grains with Solubles. Table 2 shows the highest percentage of neutral sugar from total hydrolysate of DDGS is glucose followed by xylose, glycerol, and arabinose. There is no significant difference ($p > 0.05$) between duplicate hydrolysates for glycerol, arabinose, mannose, glucose, and galactose, but there is significant difference for xylose ($p < 0.05$).

The hydrolysate of monosaccharides and LMW oligosaccharides of DDGS was mostly glycerol and glucose (Table 2). There is no significant difference between duplicate hydrolysates for arabinose, xylose, mannose, glucose, and galactose ($p > 0.05$), but there is significant difference for glycerol ($p < 0.05$). Comparison of Tables 1 and 2 shows that most of the glycerol and about half of the glucose of this distillers' product are accounted for by monosaccharides and LMW oligosaccharides.

Corn Distillers' Dried Grains. The largest percentage of neutral sugar from total hydrolysate of DDG is xylose followed by arabinose. There is no significant difference between duplicate hydrolysates of this distillers' product for glycerol, arabinose, xylose, mannose, glucose, and galactose ($p > 0.05$).

The hydrolysate of monosaccharides and LMW oligosaccharides of DDG was mostly glycerol followed by glucose. There is no significant difference between duplicate hydrolysates for arabinose, xylose, mannose, glucose, and galactose ($p > 0.05$), but there is significant difference for glycerol ($p < 0.05$). Comparison of Tables 1 and 2 indicates most of the glycerol of this distillers' product is accounted for by monosaccharides and LMW oligosaccharides.

Dowd et al. (1993) determined the low molecular weight organic composition of ethanol stillage from corn by GC–MS and HPLC. They found lactic acid, glycerol, and alanine, as well as smaller amounts of ethanol, nonnitrogenous and nitrogenous acids, polyhydroxy alcohols, sugars, and glucosides in the soluble part of corn stillage. Their results cannot be compared with our data directly, because their filtered stillage does not correspond to any of our distillers' products (our DDS contains the soluble part of stillage as well as some suspended solids). Also, we used the hydrolysates of corn distillers' products, whereas Dowd et al. (1993) worked on the filtered stillage without hydrolysis.

Conclusion. Typical calculated values for carbohydrate content are 45% for DDS, 50% for DDGS, and 53% for DDG. Our total neutral sugar contents of total hydrolysates of corn distillers' products (36–39% in Table 1) accounted for most of the calculated value for carbohydrate for each corn distillers' product. It appeared feasible to determine neutral sugar contents of corn distillers' products by HPLC after the original sample was hydrolyzed by 2 N trifluoroacetic acid. Reproducibility between replicate determinations of the same hydrolysate as well as between duplicate hydrolysates is satisfactory.

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