Neutral Sugar Contents of Corn Gluten Meal and Corn Gluten Feed

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Corn gluten meal and corn gluten feed are coproducts from wet milling of corn. To increase their potential for further processing, the carbohydrate composition of these coproducts was determined. Each material was first hydrolyzed by trifluoroacetic acid, and the noncellulosic neutral sugars were then measured by high-performance liquid chromatography. Glycerol (3.7 and 4.9%), arabinose (0 and 6.1%), xylose (0.3 and 6.3%), mannose (1.0 and 0%), glucose (7.9 and 21.1%), and galactose (0.2 and 1.7%) were found in corn gluten meal and corn gluten feed, respectively. Glycerol and glucose account for most of the neutral carbohydrates from the hydrolysis of the 80% methanol soluble fraction (monosaccharide and low molecular weight oligosaccharides).

Keywords: *Neutral sugars; corn gluten meal; corn gluten feed*

INTRODUCTION

The wet milling process involves a sulfur dioxide steep to soften the corn kernels, and milling, screening, centrifuging, and washing to produce starch, germ, fiber, and protein fractions. The steep water (after fermentation in alcohol plant) is combined with the fiber fraction to give corn gluten feed, which contains ~20% protein, 3% crude fat, 8% crude fiber, and 7% ash. The protein fraction from wet milling is corn gluten meal, which contains >60% protein, and ~1% crude fat, 1% crude fiber, and 2% ash.

Most of the starch produced from corn wet milling is converted into ethanol or sweetener, such as high fructose corn syrup. As the demands for fuel ethanol and sweetener increase, an increasing amount of corn gluten meal and corn gluten feed are also produced. Corn gluten meal and corn gluten feed have been used for animal feed. Information on the carbohydrate fraction of corn gluten meal and corn gluten feed can lead to additional uses for these materials. Although protein, fat, ash, and crude fiber contents of corn gluten meal and corn gluten feed have been reported, little is known concerning the carbohydrate fraction of corn gluten meal and corn gluten feed. Albersheim et al. (1967) found the yields of most monosaccharides from plant cell-wall preparations after trifluoroacetic acid hydrolysis are at least equal to those obtained by hydrolysis with mineral acids, and trifluoroacetic acid can be readily removed by evaporation. Albersheim et al. (1967) found that incubation with 2 N trifluoroacetic acid at 121 °C for 1 h was the optimal hydrolysis time for most of the sugars, but cellulose is not hydrolyzed under these conditions. We report on the noncellulosic neutral sugars in corn gluten meal and corn gluten feed detected after trifluoroacetic acid hydrolysis by high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Materials. Corn gluten meal and corn gluten feed were supplied by Pekin Energy Company (Pekin, IL). Corn gluten meal is a distinct high-protein fraction in the corn wet milling industry and is relatively constant in composition among different companies and among different batches from the same plant. Corn gluten feed contains fiber and steepwater (after fermentation in alcohol plant) fractions and may contain defatted corn germ. One-fourth of U.S. corn wet milling plants include defatted corn germ in corn gluten feed, but the percentage of defatted corn germ in corn gluten feed from the same plant changes with availability of corn germ from other plants that do not process corn germ and ship their corn germ to the processing plant. It is not practical to determine the neutral carbohydrate composition of corn gluten feed that contains a changing percentage of defatted corn germ, so we used corn gluten feed that does not contain defatted corn germ from Pekin Energy. Both corn gluten meal and corn gluten feed were each ground three times in an Alpine model 160Z (Augsburg, Germany) pin mill at 14 000 rpm to reduce particle size.

Hydrolysis. Inositol (5 mg) as internal standard was added to 98−116 mg of corn gluten meal and corn gluten feed before 2 N trifluoroacetic acid (2 mL) hydrolysis for 1 h at 120 °C in a 13 × 100-mm tube with a Teflon-lined screw cap in an autoclave. After hydrolysis, trifluoroacetic acid was removed completely by Bio-Rad (Richmond, CA) AG 1-X8 100−200 mesh acetate form anion exchange resin in a Pasteur pipet, and 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 at 80 °C for 20 min, and then successively with acetic anhydride (1 mL), chloroform (1 mL), and water (2 mL; Seymour et al., 1975). The material was mixed in a vortex (Scientific Products, McGaw Park, IL) for 30 s, and the chloroform layer was removed and washed with water twice and then dried. The sample (1 μL usually) was injected into a Perkin-Elmer Sigma 3B capillary chromatograph (Norwalk, CT) equipped with a Hewlett-Packard (Palo Alto, CA) cross-linked methyl silicone ultra 1 capillary column (film thickness, 0.11 μm; internal diameter, 0.2 mm; length, 25 m). The column was held at 110 °C for 3 min, and the temperature was then increased at 5 °C/min to 185 °C. Injection port and detector temperatures were 275 °C, and helium gas was the carrier. Quantitation was by peak area using inositol as the internal standard.

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

High-Performance Liquid Chromatography (HPLC). A Waters model 710B liquid chromatograph (Milford, MA) equipped with an R401 differential refractometer and an Aminex HPX-87P carbohydrate analysis column (Bio-Rad Laboratories; 300 × 7.8 mm was used at 85 °C with water as the mobile phase. The flow rate was 0.6 mL/min. The sample was hydrolyzed as described under Hydrolysis without inositol standard. Trifluoroacetic acid was removed after hydrolysis by Bio-Rad AG 1-X8 acetate form anion exchange resin, evaporated to dryness, dissolved in 2 mL of water, and filtered

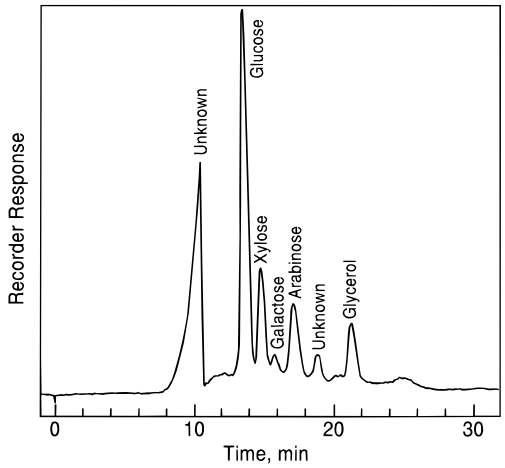

Figure 1. High-performance liquid chromatography pattern of total hydrolysates of corn gluten feed.

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 areas of standard neutral sugars.

Monosaccharides and Low Molecular Weight (LMW) Oligosaccharides. Corn gluten meal and corn gluten feed were each extracted with 80% methanol to remove monosaccharide 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 weight loss after heating at 135 °C in an air oven for 2 h (AACC, 1983). An undried sample was used for carbohydrate analysis.

Statistical Analysis. The results from duplicate hydrolysis were compared by general linear models procedure.

RESULTS AND DISCUSSION

An initial acid hydrolysis of polysaccharide into constituent monosaccharides, which are somewhat degraded by acid, is common to all methods of determining the chemical composition of polysaccharides (Dutton, 1973). The monosaccharides decomposed slower while they were in polysaccharide compared with the free monosaccharide, so a correction factor for hydrolysis losses can never be completely accurate (Biermann, 1988) and it is preferable to choose conditions of minimum decomposition. Albersheim et al. (1967) found that for most of the sugars from plant cell-wall preparations, the optimal hydrolysis time was 1 h at 121 °C in 2 N trifluoroacetic acid.

HPLC gave better results than GC in terms of reproducibility between duplicate determinations of the same hydrolysate as well as between different hydrolysates in preliminary experiments (not shown). The identity of GC peaks was confirmed by retention times and MS from the abundance versus mass/charge pattern by comparison with the known MS pattern of glycerol, arabinose, xylose, mannose, glucose, and galactose. The identities of the HPLC peaks were confirmed by comparison with GC peaks and peaks from known glycerol, arabinose, xylose, mannose, glucose, and galactose.

The HPLC pattern of total hydrolysates of corn gluten feed is shown in Figure 1 with glucose, xylose, galactose, arabinose, and glycerol peaks. (Mannose appears between arabinose and glycerol in corn gluten meal.) There was a large unknown peak that eluted around 10 min and a small unknown peak that eluted around 19 min. A large unknown peak also was present, with elution time around 10 min, from the HPLC pattern of total hydrolysate of corn distillers' grains with solubles and was shown not to be carbohydrate by the phenol–sulfuric acid test (Wu, 1994). The large unknown peak in Figure 1 is probably not carbohydrate because it has the same elution time as observed earlier and because both corn gluten feed and corn distillers' grains with solubles are derived from corn.

Corn Gluten Meal. The largest percentage of neutral sugar from total hydrolysate of corn gluten meal is glucose followed by glycerol (Table 1). There was no significant difference ($P > 0.05$) between duplicate hydrolysate of corn gluten meal for glycerol, arabinose, xylose, mannose, glucose, and galactose. It is possible that some glycerol may be derived from hydrolysis of lipids by 2 N trifluoroacetic acid with concurrent release of fatty acids. However, no fatty acid was seen in GC.

The total hydrolysate of monosaccharide and LMW oligosaccharides of corn gluten meal shows that glycerol is the predominant component, followed by glucose (Table 2). There is no significant difference between duplicate hydrolysates of corn gluten meal for glycerol,

Table 1. Neutral Sugar Contents (Percent of Dry Weights) of Total Hydrolysate of Corn Gluten Meal and Corn Gluten Feed by HPLC

sample	hydrolysate	glycerol	arabinose	xylose	mannose	glucose	galactose	total
corn gluten meal	1	3.27 (0.73)	0(0)	0.36 (0.15)	1.12 (0.44)	8.04 (0.39)	0.35 (0.19)	13.14 (1.31)
	2	4.12 (0.97)	0(0)	0.17 (0.03)	0.92 (0.18)	7.83 (0.09)	0.13 (0.04)	13.17 (1.16)
	mean	3.7 (0.9)	0(0)	0.3 (0.1)	1.0 (0.3)	7.9 (0.2)	0.2 (0.1)	13.1 (1.2)
corn gluten feed	1	5.01 (0.45)	5.84 (0.38)	6.21 (0.04)	0(0)	20.81 (0.25)[a]	1.61 (0.11)	39.48 (0.57)
	2	4.74 (0.21)	6.25 (0.43)	6.37 (0.45)	0(0)	21.31 (0.13)[a]	1.85 (0.24)	40.52 (0.44)
	mean	4.9 (0.3)	6.1 (0.4)	6.3 (0.3)	0(0)	21.1 (0.2)	1.7 (0.2)	40.1 (0.5)

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

Table 2. Neutral Sugar Contents (Percent of Original Dry Weights) of 80% Methanol Soluble Fraction Hydrolysates of Corn Gluten Meal and Corn Gluten Feed by HPLC

sample	hydrolysate	glycerol	arabinose	xylose	mannose	glucose	galactose	total
corn gluten meal	1	2.37 (1.26)	0(0)	0(0)	0.32 (0.37)[a]	1.47 (0.14)[a]	0(0)	4.16 (1.09)[a]
	2	3.60 (0.11)	0(0)	0(0)	1.01 (0.11)[a]	1.88 (0.12)[a]	0(0)	6.49 (0.12)[a]
	mean	3.0 (0.7)	0(0)	0(0)	0.7 (0.2)	1.7 (0.1)	0(0)	5.4 (0.6)
corn gluten feed	1	4.82 (0.23)	0.84 (0.45)[a]	0.17 (0.04)	0(0)	2.12 (0.01)[a]	0.25 (0.15)	8.20 (0.86)
	2	4.24 (1.03)	1.77 (0.30)[a]	0.22 (0.20)	0(0)	2.83 (0.13)[a]	0.42 (0.33)	9.48 (0.07)
	mean	4.5 (0.6)	1.3 (0.4)	0.2 (0.1)	0(0)	2.5 (0.1)	0.3 (0.2)	8.8 (0.5)

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

arabinose, xylose and galactose, ($P > 0.05$), but there is significant difference ($P > 0.05$) for mannose and glucose. Comparison of Tables 1 and 2 indicates that most of the glycerol and mannose of corn gluten meal is from monosaccharide and LMW oligosaccharides.

Corn Gluten Feed. The largest percentage of neutral sugar from corn gluten feed is glucose followed by xylose, arabinose, and glycerol (Table 1). Part of the glycerol may result from a side reaction of conversion of glucose to ethanol during fermentation (Wu, 1989). Corn gluten feed contains stillage from fermenttion of glucose to ethanol, so it will also contain glycerol. Reiners and Howland (1976) reported the average starch content of corn gluten feed was 16.7% on dry basis. Starch, after hydrolysis, becomes glucose, so starch from corn gluten feed can account for most of the 21.1% glucose reported in Table 1. There is no significant difference between duplicate hydrolysates of corn gluten feed for glycerol, arabinose, xylose, mannose, and galactose ($P > 0.05$), but there is significant difference for glucose ($P > 0.05$).

Glycerol dominates the HPLC pattern of total hydrolysate of monosaccharide and LMW oligosaccharides of corn gluten feed (Table 2). There is no significant difference between duplicate hydrolysates of corn gluten feed for glycerol, xylose, mannose and galactose ($P > 0.05$), but there is significant difference for arabinose and glucose ($P < 0.05$). Comparison of Tables 1 and 2 data shows that most of the glycerol from corn gluten feed is accounted for by monosaccharide and LMW oligosaccharides.

Nitrogen-free extract excluding cellulose of corn gluten meal is 24% and of corn gluten feed is 47% on dry basis (Wright, 1987). Our total noncellulosic neutral sugar contents of total hydrolysates of corn gluten meal (13%) and of corn gluten feed (40%; Table 1) account for most of the calculated value for carbohydrate of corn gluten meal and corn gluten feed. It seems feasible to determine neutral sugar contents of corn gluten meal and corn gluten feed by HPLC after the original sample is hydrolyzed by 2 N trifluoroacetic acid. Reproducibility between duplicate determinations of the same hydrolysates as well as between replicate hydrolysates is satisfactory. Corn gluten feed contains ~20% bound glucose, mostly from starch (Tables 1 and 2), in addition to 8% cellulose from composition data sheet of manufacturer. It may be feasible to convert the bound glucose and cellulose into free glucose and then to ethanol by yeast. The glycerol, arabinose, and xylose in corn gluten feed may also be recovered or further processed. Glucose and glycerol from corn gluten meal may also be utilized after suitable treatment.

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LITERATURE CITED

AACC. *Approved Methods*, 8th ed.; American Association of Cereal Chemists: St. Paul, MN, 1983.

Albersheim, P.; Nevins, D. J.; English, P. D.; Karr, A. A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. *Carbohydr. Res.* **1967**, *5*, 340−345.

Biermann, C. J. Hydrolysis and other cleavages of glycosidic linkages in polysaccharides. *Adv. Carbohydr. Chem. Biochem.* **1988**, *46*, 251−271.

Dutton, C. G. S. Applications of gas liquid chromatography to carbohydrates. Part I. *Adv. Carbohydr. Chem. Biochem.* **1973**, *28*, 11−160.

Reiners, R. A.; Howland, D. W. Note on vitamins and trace elements in feeds derived from the wet-milling of corn. *Cereal Chem.* **1976**, *53*, 964−968.

Seymour, F. R.; Plattner, R. D.; Slodki, M. E. Gas-liquid chromatography-mass spectrometry of methylated and deuteriomethylated per-O-acetyl-aldononitriles from D-mannose. *Carbohydr. Res.* **1975**, *44*, 181−198.

Wright, K. N. Nutritional properties and feeding value of corn and its by-products. In *Corn: Chemistry and Technology*; Watson, S. A., Ramstad, P. E., Eds.; American Association of Cereal Chemists: St. Paul, MN, 1987.

Wu, Y. V. Protein-rich residue from ethanolic fermentation of high-lysine, dent, waxy, and white corn varieties. *Cereal Chem.* **1989**, *66*, 506−509.

Wu, Y. V. Determination of neutral sugars in corn distillers' dried grains, corn distillers' dried solubles, and corn distillers' dried grains with solubles. *J. Agric. Food Chem.* **1994**, *42*, 723−726.

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