# Analysis of Neutral Carbohydrates in Agricultural Residues by Gas–Liquid Chromatography

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Agricultural residues comprise more than 50% of the material grown annually by farmers. To assess the potential of these residues, we examined a wide variety of plant residues for their neutral carbohydrate content. For this purpose we made alditol acetate derivatives and performed gas chromatography of the derivatized sugars. By this method, we determined that the xylan composition of the residues varies widely. Some of the residues, such as corn cobs and corn husks, contain approximately 30% xylose, whereas the pith of kenaf and sunflower stalks contains only 2%. The entire corn plant, kenaf stalk, wheat straw, and peanut hulls contain the largest portion of xylan in the examined residues.

Agricultural residues, the material remaining after the crop has been harvested, comprise more than 50% of the material grown annually by farmers. The stalks, leaves, and hulls are primarily disposed of by feeding to animals, plowing into the soil, or burning.

Several possible uses for these residues (Coe and Turk, 1972) that have been discussed previously are as follows: methane generation, conversion to a highly digestible ruminant feed by chemical or mixed culture fermentation (Matsuoka, 1973), and chemical or enzymic hydrolysis to sugars and alcohol production (Reese et al., 1972). The rate of hydrolysis, yield, and type of sugars recovered are dependent on the substrate source and composition. Xylans and hemicellulosic composition of soybean hulls (Sannella and Whistler, 1962; Aspinall et al., 1966) and corn cobs (Whistler and BeMiller, 1956; Whistler and Corbett, 1956; Whistler and Lauterback, 1958) have been examined previously and xylans from several annual plants (Whistler and Gaillard, 1961) have been compared.

Xylitol, a sweetener with promising characteristics, has recently drawn interest from studies suggesting that it is both anti- and noncariogenic (MaKinen, 1976). It has other unique properties that offer further applications, such as in diabetic foods and as a flavoring or flavor-enhancing agent (Scheinin and MaKinen, 1975).

Xylitol is a naturally occurring sweetener that is found in a wide variety of fruits and vegetables ranging from raspberries to cauliflower and spinach (Walshuttl, 1973). Although xylitol is widely distributed in nature, low concentrations make direct extraction impractical. Thus, for all its unique and advantageous properties, xylitol is a scarce and high-cost product.

The only current production of xylitol is from birch wood, which is rich in xylans. These xylans are hydrolyzed, and the xylose extracted from the residue is reduced to xylitol (Pintauro, 1977).

In this study a wide variety of agricultural residues have been examined for their neutral carbohydrate composition, with interest in obtaining a residue with a large concentration of xylan. If the present experimental evidence of the noncariogenic properties of xylitol is confirmed, the outlook for xylitol and any agricultural residue containing large concentrations of xylose should be enhanced.

## MATERIALS AND METHODS

In this study, a gas chromatographic procedure was used to determine the cellulosic and apparent hemicellulosic content of a series of agricultural residues by measuring the neutral carbohydrates (Sawardeker et al., 1965). The total neutral carbohydrate content was measured in one sample of a residue. In a duplicate sample, the cellulose is first isolated by extracting the noncellulosic material from the cellulose with a solution of acetic and nitric acid and the neutral carbohydrate content of the cellulose is measured. The cellulose was isolated by extracting the residue with 3 mL of an acetic–nitric acid reagent (150 mL of 80% acetic acid and 15 mL of concentrated nitric acid) in a screw-capped Teflon-lined test tube. The reagent and residue were completely mixed on a vortex mixer and heated for 0.5 h at 100 °C. The tubes were then cooled and centrifuged, and the supernatant was removed. The fibrous precipitate was washed twice with the acetic–nitric acid reagent (3 mL) and twice with acetone (2 mL). The residual acetone was removed by evaporation. The content of hemicellulose, based on the neutral aldoses, can be readily established by difference. The concentrations of the individual aldoses are calculated based on the area of the aldose and the corresponding area and weight of the internal standard, 2-deoxy-D-glucose.

The samples were collected locally, hand separated, and dried in a vacuum oven for 24 h at 100 °C. Ten to 20 plants were the source of the residues. Each sample was ground in a Wiley mill through a 40-mesh screen and stored in a desiccator until used. Each sample was analyzed in duplicate, and each duplicate was analyzed two or three times in the gas chromatograph.

The alditol acetates were prepared by a modification of the analysis procedure described previously (Sloneker, 1971). The ground samples (20–30 mg) were weighed into the 15 × 125 mm Teflon-lined screw-capped test tubes. The hydrolysis was carried out first with 72% sulfuric acid (0.3 mL) at 30 °C for 1 h, then diluted with water to 1 N sulfuric acid and placed in a preheated autoclave for 2 additional h at 120 °C. With the agricultural residues, it was found that the additional hydrolysis time was required to give more complete solution.

The sulfuric acid was then neutralized with lead carbonate and the freed aldoses were reduced to their corresponding alditols with sodium borohydride as described in the analysis procedure (Sloneker, 1971). The alditols were acetylated at 100 °C overnight with pyridine–acetic anhydride (0.2 mL of a freshly prepared 1:1 mixture). The acetylated alditols were injected without further treatment directly onto the gas chromatographic column.

The separations were carried out on a 2 m × 2 mm glass column packed with 3% ECNSS-M coated on 100–120 mesh Gas-Chrom Q (Applied Science Laboratories, State College, PA). The helium carrier gas flow rate was

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Table I. Acres Harvested and Crop Residues Produced (1977) (Agricultural Statistics, 1977, 1978)

| crop | acres harvested, millions | residue per acre (dry wt), ton/acre | residue total (dry wt), million tons |
|---|---|---|---|
| corn | 70 | 2-3 | 140-210 |
| soybeans | 57 | 1-2 | 57-114 |
| wheat | 66 | 1-2 | 66-132 |
| sorghum | 14 | 2-3 | 28-42 |
| oats | 14 | 1-2 | 14-28 |
| barley | 10 | 1-2 | 10-20 |
| cotton | 13 | 1-2 | 13-26 |
| minor crops | 15 | | 21-34 |

maintained at 40 mL/min, and the column was operated isothermally at 195 °C. The chromatographic instrument was a Packard 7400 Series (Packard Instrument Co., Downers Grove, IL). Integration of the areas under the gas chromatographic peaks was carried out via a remote hookup with a centrally located computer, a Mod Comp Model II (Butterfield et al., 1978).

Standard sugars were obtained commercially and used without further purification. One internal standard, 2-deoxy-D-glucose, was added just after the samples were autoclaved because of its relative lability in acid. Standard sugar mixtures were run with each set of determinations to minimize error due to minor variations in $R$ values caused by slight variation in hydrolysis times and conditions. The $R$ value is a response factor that is determined for each aldose from a standard aldose mixture. It is derived from the relation:

$$R = \frac{(\text{mg of std})(\text{area of aldose})}{(\text{mg of aldose})(\text{area of std})}$$

## RESULTS AND DISCUSSION

Vast quantities of agricultural residues are available every year from our cropland. These residues are presently utilized as animal feed or primarily as organic tilth. Table I illustrates the quantity of some of the agricultural residues that remain after the crops have been harvested (Agricultural Statistics, 1977, 1978). For example, 70 million acres of corn will produce about 210 million tons of residue. The annual soybean crop produces another 100 million tons of residue. From the major crops, excluding the hay crop which is already mostly fed to animals, about 350–600 million tons of agricultural residue are produced every year. From the minor crops another 20–35 million tons of residue are produced. Most of these residues, with the exceptions of sugar cane, vegetables, rice hulls, and peanuts, are left in the fields in their growing area.

Table II contains the analysis results for some of the residues examined. The carbohydrate content is broken down into the constituent sugars, as well as total aldose content and cellulose. In the wheat straw residue the pentoses arabinose and xylose are present at levels of 2.6 and 16.5%, respectively. Only trace amounts of the hexoses mannose and galactose and 31.4% glucose are present. This gives a total carbohydrate content of 51.9% and a cellulose content of 23.4%. The sunflower stalk contains 10.6% xylose and a total carbohydrate content of 40.1%. The sunflower pith, which was simply scraped from the center of the stalk, contains only about 2% xylose. Peanut hulls contain 14.9% and flax straw 10.6% xylose, respectively.

The production of soybeans yields between 1 and 2 tons of residue per acre. This residue consists of stalks and leaves and the soybean hulls. The stalks and leaves contain 11.8% xylose and the hulls only 6.1%. The hulls also contain 6.6% galactose, the highest amount of any of the residues that were examined.

Kenaf is a new crop that is being examined as a replacement crop for the production of newsprint (Bagby, 1977). Although it is not listed in Table I, it does produce between 10 and 20 tons of dry matter per acre. The bark and pith of the plant contain only 7.7 and 2.4% xylose, respectively. However, the stalk, which is the major portion of the plant, contains 14.1% xylose and only trace amounts of arabinose, mannose, and galactose.

The results of the corn residue analyses are given in Table III. These residues amount to between 2 and 3 tons per acre.

Table II. Neutral Carbohydrate Content of Agricultural Residues

| plant residue | percent of total sample weight | | | | | | |
|---|---|---|---|---|---|---|---|
| | arabinose | xylose | mannose | galactose | glucose | total aldose | cellulose |
| wheat straw | 2.6 | 16.5 | 0.5 | 0.9 | 31.4 | 51.9 | 23.4 |
| sunflower stalk | 0.4 | 10.6 | 0.4 | 0.4 | 28.3 | 40.1 | 22.6 |
| sunflower pith | 2.1 | 1.9 | 1.2 | 1.3 | 25.1 | 31.6 | 13.8 |
| peanut hulls | 1.6 | 14.9 | 0.3 | 0.4 | 42.8 | 60.0 | 27.8 |
| flax straw | 2.1 | 10.6 | 1.3 | 2.2 | 34.7 | 50.9 | 34.5 |
| sweet clover hay | 3.2 | 7.2 | 1.2 | 1.7 | 31.1 | 44.4 | 29.8 |
| soybean stalks and leaves | 1.3 | 11.8 | 3.1 | 2.3 | 32.1 | 50.6 | 33.3 |
| soybean hulls | 2.9 | 6.1 | 2.5 | 6.6 | 21.1 | 39.2 | 16.3 |
| kenaf bark | 2.1 | 7.7 | 1.4 | 1.5 | 34.1 | 46.8 | 33.1 |
| kenaf pith | 1.5 | 2.4 | 0.8 | 1.9 | 31.0 | 37.6 | 7.9 |
| kenaf stalk | 0.3 | 14.1 | 0.8 | 0.4 | 36.0 | 51.6 | 31.4 |

Table III. Neutral Carbohydrate Content of Corn Plant Residues

| plant residue | percent of total sample weight | | | | | | |
|---|---|---|---|---|---|---|---|
| | arabinose | xylose | mannose | galactose | glucose | total aldose | cellulose |
| corn cobs | 4.6 | 31.2 | trace | trace | 41.7 | 77.5 | 29.6 |
| corn leaves | 3.0 | 18.7 | 0.3 | 0.5 | 31.0 | 53.5 | 21.7 |
| corn stalks | 2.4 | 18.9 | 0.6 | 1.1 | 35.9 | 58.9 | 32.0 |
| corn husks | 6.8 | 29.6 | 0.3 | 0.6 | 45.1 | 82.4 | 27.1 |
| corn pith | 2.3 | 16.8 | 0.3 | 0.4 | 38.4 | 58.2 | 34.7 |
| corn fiber | 1.9 | 18.3 | 0.5 | 0.3 | 42.1 | 63.1 | 34.4 |

The corn cob contains large quantities of glucose and xylose, 41.7 and 31.2%, respectively, with only 4.6% arabinose and trace amounts of mannose and galactose. The husks surrounding the ear of corn contain almost as much xylose as the cob: 29.6%. The leaves and stalk of the corn plant both contain more than 18% xylose. The entire corn plant contains large quantities of xylose and glucose, between 2 and 7% arabinose, and only small amounts of mannose and galactose.

The corn pith and corn fiber (Table III) are the products of air classification of the center portion of the corn stalk (Jones et al., 1979). This material was scraped from the corn stalk, ground, and then submitted to a gentle air stream. The fiber was the more dense material and remained behind. These materials differed little in composition.

A variety of plant residues have been examined for their neutral carbohydrate composition to determine the possible utility of the residue. Certainly the corn plant residue is the richest in the xylans, with xylose contents ranging from 19% of the stalk and leaves to 30% of the cobs and husks. Also, the 2–3 tons per acre of residue make the corn crop most appealing for the production of xylitol. Another very promising crop would be the kenaf crop; although containing only 15% xylose, the plant does produce between 10 and 20 tons of material per acre annually. These residues are excellent sources for the recovery of xylose, leading to the production of xylitol, a potential noncariogenic sweetener.

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# Glass Capillary Gas Chromatography for Quantitative Determination of Volatile Constituents in Cold-Pressed Grapefruit Oil

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Thirty-two components of Florida cold-pressed grapefruit oil were separated on a 30-m glass capillary column coated with Carbowax 20M. They were quantitated on the basis of both normalization and internal standard methods by use of a microprocessor-controlled GC terminal. Compounds not previously quantitated for grapefruit oil were β-pinene, *cis*- and *trans*-limonene oxides, citronellyl acetate, octanol, humulene, and carvone. Knowledge of their presence is important, since their individual contributions to the overall flavor profile of grapefruit oil can then be determined.

Quantitative analyses of cold-pressed citrus oils are becoming increasingly important, particularly in relation to quality control methods, chemotaxonomy of hybrid fruit, and those natural insect attractants or toxicants present in the oils. The need for better techniques to obtain reliable quantitative information on cold-pressed citrus oils is readily apparent from the widely different quantitative values that have been reported (Shaw, 1979). Recent developments in column technology and instrumentation for glass capillary gas chromatography have made it the current method of choice for obtaining reliable quantitative values for individual components of cold-pressed citrus oils.

Considerably less has been reported regarding the quantitative analysis of grapefruit oils than of the other major citrus oils (Shaw, 1979). Early methods for quantitating grapefruit oil [reviewed by Shaw (1979)] usually involved gas chromatography on packed columns. However, in two studies on grapefruit aldehydes, citral was determined colorimetrically (Yokoyama et al., 1961) and

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