MINOR SACCHARIDES (Gas-Liquid Chromatography)

PRINCIPLE

The saccharides are converted to their trimethylsilyl (TMS) ethers which are separated by gas-liquid chromatography (GLC). By using suitable columns, liquid phases, solid supports, and temperature programs, the mono-, di-, tri-, and tetrasaccharides may be determined. The principal non-dextrose sugars are the disaccharides maltose, isomaltose, and gentiobiose.

SCOPE

The method is applicable to crude and refined corn sugars, including dextrose and high dextrose starch hydrolyzates.

SPECIAL APPARATUS

1. Gas Chromatograph: A modern gas chromatograph equipped with a flame ionization detector, a suitable injection system and a suitable data acquisition system is recommended.

2. Gas Chromatograph Columns: Stainless steel, 6 ft., 1/8 in., packed with 80-100 mesh silanized Chromosorb W coated with 1% JXR or 2% OV-17 (Note 1), or equivalent capillary columns.

3. Test Tubes: With Teflon lined screw cap, e.g., Kimble No. 45066-A 16 × 125 mm

REAGENTS

1. Pyridine: ACS reagent grade

2. Hexamethyldisilazane: Specially purified grades are available from Applied Science Laboratories, Inc., P. O. Box 440, State College, Pennsylvania 16801 or the Pierce Chemical Company, P.O. Box 117, Rockford, Illinois 61105.

SACCHARIDES (Gas-Liquid Chromatography) — continued

3. Trifluoroacetic Acid, 99%: Available from the Pierce Chemical Company

4. Phenyl Glucoside Solution, Internal Standard: Weigh accurately about 30 mg of phenyl β-D-4-glucopyranoside (Pierce Chemical Company) and transfer to a 50 mL volumetric flask; add 25 mL pyridine, shake until the sugar dissolves and dilute to volume with pyridine.

5. Standard Sugar Solution (Note 2): Weigh accurately about 60 mg of maltose hydrate (calculate as anhydrous maltose) and about 60 mg of gentiobiose and transfer to a 100 mL volumetric flask; dissolve in purified water and dilute to volume. Mix thoroughly and pipet 1 mL into a 16 × 125 mm test tube; place the tube in a water bath at 60 °C and remove the water under reduced pressure. Weigh about 100 mg of NIST (National Institute of Standards and Technology) dextrose and transfer to the tube containing the maltose and gentiobiose. Pipet 1 mL of the phenyl glucoside solution into the tube, cap, and warm the mixture in 60-70 °C water bath until the dextrose dissolves.

INSTRUMENT PARAMETERS

The following applies to a packed column:

1. Column Oven Temperature: Initial oven temperature 150 °C; raise temperature 10 °C per min. to 325 °C and hold at this temperature until all tetrasaccharides are eluted (requires 6-8 mins. after reaching 325 °C).

2. Injection Port and Block Temperature: 300 °C

3. Gas Flows: Carrier gas, helium, 40 psi, 35 cc/min.; hydrogen, 8 psi, 35 cc/min.; air, 300-400 cc/min.

PROCEDURE

Standardization: Add 0.9 mL hexamethyldisilazane and 0.1 mL of trifluoroacetic acid to the standard sugar solution. Cap the tube and shake for 30 secs.; let stand 30 mins. with occasional shaking. Properly prepared derivative solutions should

SACCHARIDES (Gas-Liquid Chromatography) — continued

be clear. If the solution is not clear, warm for 5-10 mins. at 60 °C. No precipitate is formed during this method of derivatization. The sample is now ready for the gas chromatographic separation.

Inject a 1 µL aliquot of the derivatized sample into the gas chromatograph. Hold the column temperature at the upper limit (325 °C), long enough to elute a tetrasaccharide. Return the column temperature to 150 °C. Record the areas of the internal standard and saccharide peaks.

Sample Analysis: Weigh accurately 100-125 mg of sample dry substance and transfer to a 16 × 125 mm test tube; add 1 mL of the phenyl glucoside solution and warm in a 60 °C bath until the sample dissolves. Proceed with the derivatization and gas chromatographic separation as described under Standardization. Record the areas of the internal standard and other saccharide peaks (Note 3).

CALCULATIONS

Standard: From the sample weights and peak areas of the sugars in the standard, determine the detector response (K value) for each sugar, e.g., for maltose:

$$\text{K (Maltose)} = \frac{\text{Total Peak Area for Maltose/Peak Area of Internal Std.}}{\text{Wt. of Maltose, mg anhydrous/Wt. on Internal Std., mg}}$$

Sample: The amount of maltose in an unknown is then calculated from the maltose peak area, the internal standard peak area, and the K value:

$$\text{Weight of Maltose in Sample (mg)} = \frac{(\text{Wt. of Internal Std., mg})(\text{Peak Area of Maltose in Sample})}{(\text{K, Maltose})(\text{Peak Area of Internal Std.})}$$

$$\%\ \text{Maltose in Sample (dry basis)} = \frac{(\text{Maltose in Sample, mg})(100)}{\text{Sampley Substance, mg}}$$

Concentrations of other saccharides in the sample are calculated in a similar manner.

NOTES AND PRECAUTIONS

1. The column should be conditioned overnight at 350 °C, using a carrier gas flow of 30 cc/min.

SACCHARIDES (Gas-Liquid Chromatography) — continued

2. Maltose hydrate, grade HHH, Hayashibara Company, Ltd., Okayama, Japan, is satisfactory and contains about 1% impurities, chiefly maltotriose. Gentiobiose may be obtained from Nutritional Biochemicals, Cleveland, Ohio 44128.

3. Isomaltose and gentiobiose are not completely separated when a packed column is used, in that gentiobiose is eluted in the leading edge of the β-isomaltose peak. However α-isomaltose, β-isomaltose, and gentiobiose have essentially the same K value so that the isomaltose content may be calculated using the K value obtained from the gentiobiose standard. The isomaltose content alone may be estimated from the α-isomaltose peak and the gentiobiose may be corrected for the interfering β-isomaltose by use of the appropriate anomeric proportionality factor.

METHOD HISTORY

Corn Sugars, Saccharides (Gas Liquid Chromatography) (F50), Date of Acceptance 9-20-1971, Revised 3-01-1995.