SACCHARIDES (Gas-Liquid Chromatography)

PRINCIPLE

The mono-, di-, tri- and other saccharides in corn syrup are converted to their corresponding oximes, and then to their volatile trimethylsilyl ethers for separation by gas-liquid chromatography. Quantitation is achieved by flame ionization detection in the presence of phenyl ß-D-glucopyranoside internal standard (Note 1).

SCOPE

The method is applicable to corn syrups, high dextrose starch hydrolyzates, high fructose corn syrups, corn sugars, sucrose and any blend thereof. Oxime preparation may be omitted in the absence of fructose and sucrose (Note 2).

SPECIAL APPARATUS

1. Gas Chromatograph: A single or dual column instrument, temperature programmable to 350 °C, equipped with flame ionization detector and computing recording integrator.

2. Sampling Microsyringe: 10 µL capacity, such as Hamilton 701N, or equivalent.

3. Test Tubes: 16 x 125 mm, with TELFON lined screw caps, such as Kimble No. 45066-A, or equivalent.

4. Rotary Evaporator: Buchi, or equivalent, equipped with 50 mL round bottom flasks.

REAGENTS

1. Gas Liquid Chromatographic Column: Stainless steel, 6 ft. x 1/8 inch, packed with 3% OV-101 on 100-120 mesh Chromosorb W(HP) (Note 3).

2. Phenyl ß-D-Glucopyranoside: Available from Nutritional Biochemicals Corporation, Cleveland, OH.

3. Hydroxylamine Hydrochloride: Analytical Reagent Grade.

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4. Pyridine: ACS Reagent Grade.

5. Dimethylaminoethanol.

6. Internal Standard and Oximation Reagent (Note 4): Weigh accurately 150 mg of ß-D-glucopyranoside and 1.00 g of hydroxylamine hydrochloride; transfer to a 100 mL volumetric flask, add 50 mL pyridine, shake to dissolve the glucoside and hydroxylamine hydrochloride, dilute to volume with pyridine and mix thoroughly.

7. Buffered Internal Standard and Oximation Reagent: Immediately before use, mix 20.0 mL of reagent 6 (above) with 1 mL of dimethylaminoethanol (Note 5).

8. Hexamethyldisilazane: Available from Pierce Chemical Co., P.O. Box 117, Rockford, IL 61105.

9. Trifluoroacetic Acid, 99%: Available from Pierce Chemical Co., P.O. Box 117, Rockford, IL 61105.

10. Saccharides for Standardization: D-Fructose, NRC-Pfanstiehl Laboratories, Inc., Waukegan, IL; D-Glucose and Sucrose, National Institute of Standards and Technology, Gaithersburg, MD; Maltose Hydrate, Grade HHH, Hayashibara Biochemical Laboratories, Inc., 2-3, 1-CHOME, Shimoishii, Okayama, Japan; other saccharides may be prepared by preparative paper, cellulose column or high performance liquid chromatography. A standard mixture is prepared by mixing the pure saccharides by weight to simulate the test sample composition as closely as possible (Note 6).

CHROMATOGRAPH PARAMETERS

1. Column Oven Temperature: Inject sample at 100 °C and immediately raise oven temperature to 300 °C at a rate of 8 °C per min.; hold at 300 °C for 10 mins. to clear column (Note 7).

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2. Injector Port Temperature: 300 °C.

3. Detector Temperature: 325 °C.

4. Gas Flow Rates: Helium - 40 mL/min.; Hydrogen - 30 mL/min.; Air - 350-400 mL/min. (Note 7).

PROCEDURE

Standardization: Pipet 5.0 mL of an appropriate aqueous standard solution containing about 300 mg saccharides per 100 mL (Note 6) into a 50 mL round bottom flask, place in rotary evaporator and evaporate to dryness in water bath at 60 °C. Add 3.0 mL of buffered internal standard oximation reagent, stopper and shake until solution is complete. Place flask in air oven (or water bath) at 70 °C for 30 mins. Cool to room temperature, add 1.0 mL of hexamethyldisilazane and 0.1 mL of trifluoroacetic acid. Stopper and shake to mix contents of flask; let stand 30 mins. Inject a 1 µL aliquot of the derivatized sample into the gas-liquid chromatograph and start the temperature program immediately. Complete the chromatographic cycle; observe and record the areas of the internal standard and saccharide peaks.

When the oximation step is omitted (Note 2), dissolve 150 mg of phenyl ß-D-glucopyranoside in pyridine, dilute to 100 mL with pyridine, and add 3.0 mL of this internal standard solution to the standard saccharide mixture residue after evaporation. Then proceed with trimethylsilyl ether preparation and chromatography as outlined above.

Sample Analysis: Weigh accurately about 15 mg of test sample dry substance (Note 8) in a 16 x 125 mm test tube. Add 3.0 mL of buffered internal standard and oximation reagent, cap the test tube, shake to complete solution and proceed as described under standardization. Observe and record the areas of the internal standard and saccharide peaks.

CALCULATION

The detector response factor (K value) is calculated for each saccharide based on the amount (mg) present in the standard saccharide mixture, the amount of internal standard (mg) added, and the respective peak areas. Each K value is then used to correct the weight (mg) of each saccharide obtained from the ratios of

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peak areas and internal standard weight (mg) for the test sample. The example below, for fructose, is applicable to all saccharides (Note 9).

$$K_F = \frac{(\text{Fructose Peak Area})/(\text{Internal Standard Peak Area})}{(\text{Fructose Wt. (mg)})/(\text{Internal Standard Wt (mg)})}$$

Fructose Weight (mg) in Test Sample =

$$\frac{\text{Internal Standard Wt. (mg)} \times \text{Fructose Peak Area}}{K_F \times \text{Internal Standard Peak Area}}$$

$$\text{Fructose, \% (Dry Basis)} = \frac{\text{Fructose in Sample (mg)} \times 100}{\text{Sample Dry Substance Wt. (mg)}}$$

NOTES AND PRECAUTIONS

1. Reaction of the reducing ends of the saccharides with hydroxylamine effectively eliminates the multiple peaks of the trimethylsilyl ethers of the dominating forms of the anomers of the mono-, di- and trisaccharides. This is especially a problem with test samples containing fructose, with 4 out of 5 possible anomers present in significant amounts. Glucose and maltose exhibit only 2 anomeric trimethylsilyl derivatives each, and acid converted and dual conversion corn syrups may be analyzed without prior oxime formation. However, even in the absence of fructose, sucrose will interfere by coelution with other derivatized disaccharide peaks.

2. The method performed with or without oxime preparation, does not allow for the separation of psicose. Maltose and isomaltose in corn syrups are well resolved. A disaccharide having the same retention time as gentiobiose has been observed in some dual conversion corn syrups when the method is performed without oxime preparation; it elutes between the tail end of the silylated ß-maltose peak and the silylated isomaltose peak. For information on trace glucosaccharides in acid converted corn syrups, see L. D. Ough, *Anal. Chem.* 34, 660 (1962).

3. The following columns also have been found satisfactory for separating the trimethylsilyl ethers of the saccharide oximes:

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a. 2% OV-17 on 100-200 mesh Chromosorb W

b. 3% SP-2100 on 100-120 mesh Chromosorb W

For separating the trimethylsilyl ethers of the free saccharides, the following column has been recommended:

c. 10% SE-52 on 60-80 mesh silanized Chromosorb W

Column c has operating parameters different from those of a and b due to its coarser particle size. See Note 7 below. On the other hand, no single set of conditions for any given column allows for all specific separations desired. Additional information on the preparation and separation of the trimethylsilyl ethers of carbohydrates may be found in the references given below.

4. Methyl α-D-mannopyranoside, also has been recommended as an internal standard by J. S. Sawardeker, J. H. Sloneker, *Anal. Chem*. 37, 945 (1965). Like phenyl ß-D-glucopyranoside, it may be dissolved in pyridine with or without added hydroxylamine hydrochloride or trimethylaminoethanol, depending on whether the method is performed with or without oxime preparation.

5. Use of the trimethylaminoethanol buffer is mandatory in the analysis of samples containing sucrose (P.G. Morel DuBoil and K. J. Schaffler, *Proceedings of the South African Sugar Technologists' Association*, June, 1978, pp. 1-10), to prevent hydrolysis to glucose and fructose producing false high values for these saccharides. Buffered hydroxylamine reagent cannot be stored for extended periods of time. Discard reagent after 1 month.

6. A preanalyzed product sample may be used as a secondary standard for a frequently analyzed sample type. It should be stored frozen in several small vials, and should be thawed and used completely for one standardization.

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7. When analyzing the saccharide silyl ethers without preliminary oximation, use the 10% SE-52 on 60-80 mesh silanized Chromosorb W column and the following instrument parameters:

   Column Oven Temperature: Inject sample at 100 °C and immediately raise oven temperature to 300 °C at a rate of 4 °C per min.; hold at 300 °C for 10 mins. to clear column.

   Injector Port Temperature: 300 °C.

   Detector Temperature: 325 °C.

   Gas Flow Rates: Helium - 75 mL/min.; Hydrogen - 30 mL/min.; Air - 500-700 mL/min.

8. A larger quantity of sample may be taken if desired by using the following technique: weigh accurately about 1.5 g sample dry substance, transfer quantitatively to a 500 mL volumetric flask, dilute to volume with purified water and mix thoroughly. Pipet 5 mL of this solution into a 50 mL round bottom flask, evaporate to dryness and proceed as described under Standardization.

9. Fructose and psicose are not separated by this technique.

REFERENCES

C. C. Sweeley, R. Bentley, M. Makite and W. W. Wells, *J. Am. Chem. Soc*., 85, 2947 (1963)

K. M. Brobst and C. E. Lott, Jr., *Cereal Chem*., 43, 35 (1966)

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