FERMENTABLE SUGARS

PRINCIPLE

Saccharides are converted to their trimethylsilyl (TMS) ethers in pyridine solution. Dextrose, maltose and maltotriose are separated on a gas chromatographic column, and their concentrations are measured by a flame ionization detector.

SAFETY

Person(s) performing this method should be trained in the handling and disposal of organic reagents/solvents, in particular, in avoiding inhalation and skin contact of pyridine, trifluoroacetic acid and hexamethyldisilazane. They should wear appropriate apparel, eye and hand protection. Chromatographic column packing and silylation reaction require use of well-ventilated hood. Analysts should also review the Manufacturing Safety Data Sheets (MSDS) for all of the chemicals required, however this review is not a substitute for adequate training.

SCOPE

The method is applicable to determination of fermentable sugars in commercial corn syrups and other starch hydrolyzates which contain essentially dextrose and its polymers (Note 1).

SPECIAL APPARATUS

1. Gas Chromatograph: Hewlett-Packard Model 5750 or equivalent, equipped with flame ionization detector.

2. Gas Chromatograph Column: Stainless steel, 6 ft. x 1/8 inch, packed with 3% OV-17 phenyl metyl silicone (50% phenyl) on 80-100 mesh silanized Chromosorb W diatomite support.

3. Syringe: Hamilton No. 701N W/G, 10-µl capacity, or equivalent.

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REAGENTS

1. Phenyl β-D-Glucopyranoside: Internal standard. Available from ICN Pharmaceuticals, Inc., 26201 Miles Road, Cleveland, Ohio.

2. Standard Sugars: Dextrose, maltose hydrate and maltotriose. Weigh portions of each to approximate the amounts anticipated in the sample (Note 3). Recommended suppliers for sugar standards are as follows: dextrose (National Institute of Standards & Technology); maltose hydrate (Sigma Ultra 99% Sigma/Aldrich Chemical Co. St. Louis, MO); maltotriose (Sigma/Aldrich), or equivalent.

3. Hexamethyldisilazane: Available from Regis Chemical Company, or equivalent.

4. Trifluoroacetic Acid: Eastman No. 6287, or equivalent.
5. Pyridine, Anhydrous: Reagent grade.

INSTRUMENT PARAMETERS

1. Column Oven Temperature: Initial oven temperature 140 °C. Hold for 3 minutes, then increase temperature at 10 °C/minute to a maximum of 330 °C, and hold for at least 4 minutes.

2. Injector Temperature: 330 °C.

3. Detector Temperature: 350 °C.

4. Detector: Hydrogen flame ionization detector with hydrogen flow rate of 35 mL per minute and air flow rate of 300 mL/minute.

5. Carrier Gas: Helium. Using a flow rate of 30 mL/minute, adjust reference flow rate to provide a flat base line.

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PROCEDURE

The required precision of weighing is indicated by the number of decimal digits.

Weigh accurately 1.5 g (as is) of sample, or the appropriate amounts of standard sugars (Note 3), and 200 mg of phenyl glucoside internal standard into a 50 mL beaker. Add 10-15 mL of pyridine and place the beaker on a steam bath; mix the contents with a glass stirring rod until the syrup dissolves. Transfer the solution quantitatively with the aid of additional pyridine into a 25 mL volumetric flask; cool, and dilute to volume with pyridine. Pipet (Note 2) a 3 mL aliquot into a 25 mL volumetric flask, and add 3.0 mL of hexamethyldisilazane and 0.3 mL of trifluoroacetic acid, leaving the flask unstoppered. Allow the reaction to subside, then mix contents of the flask by swirling. Add two 4 mm glass beads and boil for 15 minutes on a hot plate, using the lowest temperature which maintains boiling. Cool and dilute to volume with pyridine; the volume of the beads may be ignored. Remove a 3 µL aliquot and inject it into the chromatograph, and start the temperature program cycle immediately. Adjust attenuation to provide optimum sensitivity, as determined by trial run. When the maltotriose has been eluted, determine areas under the various peaks by a suitable method, and correct them for the respective attenuation values.

CALCULATION

Divide the corrected area (A) of each peak in the standard chromatogram (representing the three sugars and phenyl glucoside internal standard) by the weight (Note 3) of the corresponding component in the standard mixture, to obtain their unit responses. Divide the unit responses for dextrose, maltose, and maltotriose by that for phenyl glucoside to obtain the relative response factors, K, for the sugars with respect to the internal standard.

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Calculate the concentration of each sugar in the sample; use the corrected peak areas of the sugar and of phenyl glucoside in the sample chromatograms, the K value for the sugar, and the weight of phenyl glucoside (PG) which was added to the sample:

$$\% \text{ Dextrose (as is)} = \frac{\text{PG Wt. (mg)} \times 100}{A_{PG} \times \text{Sample Wt. (g)} \times 1{,}000} = \frac{A_{dextrose}}{K_{dextrose}}$$

$$\% \text{ Maltose (as is)} = \frac{\text{PG Wt. (mg)} \times 100}{A_{PG} \times \text{Sample Wt. (g)} \times 1{,}000} = \frac{A_{maltose}}{K_{maltose}}$$

$$\% \text{ Maltotriose (as is)} = \frac{\text{PG Wt. (mg)} \times 100}{A_{PG} \times \text{Sample Wt. (g)} \times 1{,}000} = \frac{A_{maltotriose}}{K_{maltotriose}}$$

% Fermentable Sugars = % Dextrose + % Maltose + % Maltotriose

NOTES AND PRECAUTIONS

1. Fermentation of corn syrups and other starch hydrolyzates by baker's yeast under the conditions of Corn Refiners Association's Standard Analytical Method E-28 appears to be limited to dextrose, maltose and maltotriose, and fermentation of these sugars is essentially complete. However, the sum of the concentrations of the three sugars in a syrup is slightly greater than its fermentables content as determined by CRA-SAM E-28 because the unfermented residue contains glycerol formed during fermentation. Experiments with typical products indicate that values of fermentables (% dry basis) according to method E-28 may be approximated by subtracting 2.9 from the sum of dry basis concentrations of dextrose, maltose and maltotriose.

2. It is essential that the standard composition should approximate that of the sample, to avoid error resulting from adsorption of the components on the chromatographic column.

3. For calculation purposes, the weight of standard maltose hydrate should be multiplied by 0.95, so that maltose content of secondary standard syrups and of samples will be expressed as anhydrous maltose.

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REFERENCE

Methods of Analysis of the American Society of Brewing Chemists, Eighth Revised Edition, Adjunct Materials, Sugars and Syrups – 17: Fermentable Saccharides by Chromatography, pp. 1-4 (1992).

METHOD HISTORY

Corn Syrup, Fermentable Sugars, GC (E-29), Date of Acceptance 4-28-78, Revised 4-28-2001.