DEXTROSE

PRINCIPLE

The method is based on the enzymatic oxidation of d-glucose (dextrose) in the presence of glucose oxidase to form hydrogen peroxide which reacts with a dye in the presence of horseradish peroxidase to give a colored product proportional to the dextrose concentration.

SCOPE

The method is applicable to all corn syrups and all starch hydrolyzates prepared by acid and/or enzyme conversion. The method can be automated by sample injection through membrane bound glucose oxidase in an analyzer equipped with a hydrogen perioxide detector (Note 1).

SPECIAL APPARATUS

1. Double Beam Spectrophotometer: An instrument capable of accurate absorbance measurements at 540 nm and equipped with a matched pair of 1 cm cuvettes.

2. Water Bath: Capable of maintaining a temperature of 30 ± 1 °C.

REAGENTS

1. Glucose Test Solution:

   Glucose Oxidase: 1000 glucose oxidase units/mL; purified (Miles Laboratories, Inc., or equivalent)

   Horseradish Peroxidase: Available from Worthington Biochemical Co., Halls Mill Road, Freehold, NJ 07728

   Chromogen: *o*-Dianisidine•2HCl

   Acetate Buffer Solution: pH 5.5, 0.1 *M*

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Dissolve 13.608 g sodium acetate trihydrate ($Na(C_2H_3O_2)_2 \bullet 3H_2O$) and dilute to 1 L with purified water. Add 2.7 mL acetic acid and adjust pH to 5.5 with sodium acetate or acetic acid, if necessary. Dissolve 40 mg chromogen, 40 mg horseradish peroxidase, and 0.4 mL glucose oxidase in 0.1 *M* acetate buffer and dilute to 100 mL with buffer solution.

2. Sulfuric Acid Solution: Add 250 mL concentrated sulfuric acid to purified water and dilute to 1 L.

3. Standard D-Glucose (Dextrose) Solution: Dissolve, in purified water, 1.000 g NIST anhydrous dextrose, previously dried at 70 °C under vacuum for 4 hrs. Dilute to 1 L in a volumetric flask. Allow to stand for 2 hrs. to permit mutarotation to occur (Note 2). Prepare fresh daily.

PROCEDURE

Standardization: Pipet 2.0, 4.0, 6.0 and 8.0 mL of the standard dextrose solution into respective 100 mL volumetric flasks. Dilute to volume with purified water and mix thoroughly.

Pipet 2.0 mL of each of the standards (respectively, 0.04, 0.08, 0.12 and 0.16 mg of dextrose) into separate 18 × 150 mm test tubes. Add 2.0 mL of purified water to a similar test tube for the reagent blank.

Equilibrate all test tubes to 30 °C in the constant temperature water bath for 5 mins. Add 1.0 mL glucose test solution to each of the test tubes allowing a 30-60 sec. interval between additions. Mix the solutions in the test tubes thoroughly, replace in the water bath and allow to remain for exactly 30 mins. Remove the samples from the bath (at 30-60 sec. intervals) and immediately add 10 mL sulfuric acid solution to each tube to stop the reaction. Mix thoroughly after addition of the acid and cool to room temperature. Determine the absorbance of the solution against the reagent blank at 540 nm using 1 cm cuvettes.

Prepare a calibration curve by plotting the absorbance of the dextrose standards against mg dextrose on linear coordinates.

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Analysis: Determine the sample dry substance by an approved method. Accurately weigh a sample into a weighing bottle equipped with a cover. Transfer quantitatively to a volumetric flask and dilute with purified water to a volume such that a 2 mL aliquot will contain between 0.10 and 0.15 mg of dextrose. For regular corn syrup, use approximately 4 g of sample diluted to 500 mL in a volumetric flask. Prepare a second dilution of 5 mL to 100 mL and use a 2 mL aliquot for analysis. Treat the sample exactly as described under Standardization, beginning with the second paragraph. Obtain the dextrose content of the sample by a reference to the standardization curve.

CALCULATION

$$\% \text{ Dextrose (dry basis)} = \frac{(\text{mg dextrose in sample from graph})(100)}{\text{Sample Wt., mg dry substance}}$$

NOTES AND PRECAUTIONS

1. A YSI 2700 SELECT Biochemistry Analyzer, or equivalent, equipped with autosampler or continuous monitoring accessory is recommended. The YSI instrument (YSI Inc., Yellow Springs, OH 45387) can also accommodate a bound glucose oxidase membrane containing mutarotase, to accelerate mutarotation of dissolved solid dextrose. Standardization is achieved by means of pure dextrose solutions of known concentrations and measurement is carried out after sample dilution in the range of linear response of the instrument.

2. Complete mutarotation is important because glucose oxidase enzyme is specific for ∃-D-glucose.

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REFERENCE

1. Association of Official Analytical Chemists, *Official Methods of Analysis (1990)*, 15th Edition, Method 969.39, "Glucose in Corn Sirups and Sugars, Glucose Oxidase Method, First Action 1969, Final Action 1970," p. 1042.

METHOD HISTORY

Corn Syrup, Dextrose, Enzamatic (E-24), Date of Acceptance 11-13-1967, Revised 10-24-1994.