TOTAL SUGARS

PRINCIPLE

A dilute starch molasses (Note 1) solution is hydrolyzed with mineral acid, converting the bulk of the oligosaccharides to dextrose. The total reducing sugars are then determined by the Lane and Eynon copper reduction procedure using Fehling's Solution. The amount of copper reduced is proportional to the total reducing sugars in the hydrolyzed sample. The total reducing sugars are expressed as dextrose which is used for reagent standardization (Note 2).

SCOPE

The method is applicable to all starch hydrolyzates prepared by acid or enzyme conversion and combinations thereof. The method is intended specifically for the analysis of starch molasses.

SPECIAL APPARATUS

1. Hydrolysis Assembly: Support a heating mantle (Glas-Col 335 watt for 1 L flask, No. 11-471-10C, Fisher Scientific Company or equivalent) in a ring support attached to a ringstand. Connect the heating mantle to a variable transformer (POWERSTAT, 10 ampere, No. 9-521-5, Fisher Scientific Company or equivalent). Place in it a 1 L, 3-necked, distilling flask, side necks Ts 24/40, center neck Ts 34/45 (No. 10-164B, Fisher Scientific Company or equivalent). Attach a reflux condenser (Allihn type, Ts 24/40, No. 7-734B, Fisher Scientific Company or equivalent) to one of the side necks and mount a thermometer, -20 to 110 °C, in the other. Close the center neck with a rubber stopper.

2. Titrating Assembly: Mount a ring support on a ringstand 1-2 ins. above a gas burner and a second ring 6-7 ins. above the first. Place a 6 in. open wire gauze on the lower ring to support a 200 mL Erlenmeyer flask and a 4 in. watch glass with center hole on the upper ring to deflect heat. Attach a 25 mL buret to the ringstand so that the tip just passes through the watch glass centered above the flask (funnel top buret with diagonal TEFLON

TOTAL SUGARS — continued

Plug, KIMAX No. 17055F recommended). Place an indirectly lighted white surface behind the assembly for observing the end point.

REAGENTS

1. Sulfuric Acid Solution, 4 *N*: Cautiously and with continuous stirring, add 204 g concentrated sulfuric acid (96% H_2SO_4; sp g 1.84) to about 700 mL purified water. Cool to room temperature and dilute to 1 L.

2. Sodium Hydroxide Solution, 50% (w/w): Add 100 mL purified water to 100 g reagent grade sodium hydroxide pellets and stir until solution is complete.

3. Sodium Hydroxide Solution, 0.1 *N*

4. Fehling's Solution:

   A. Dissolve 34.64 g of reagent grade crystalline copper sulfate pentahydrate ($CuSO_4 \bullet 5H_2O$) in purified water and dilute to 500 mL volume.

   B. Dissolve 173 g of reagent grade potassium sodium tartrate tetrahydrate ($KNaC_4H_4O_6 \bullet 4H_2O$) and 50 g of reagent grade sodium hydroxide (NaOH) in purified water and dilute to 500 mL volume.

   Measure a quantity of Solution A, add an equal quantity of Solution B, and mix (Note 3). Standardize as follows, immediately prior to use:

   Dry a quantity of National Institute of Standards and Technology (NIST) dextrose in a vacuum oven at 70 °C for 4 hrs. Dissolve 3.000 g in purified water, dilute to 500 mL volume and mix thoroughly. Pipet 25.0 mL of mixed Fehling's Solution into a 200 mL Erlenmeyer flask that contains a few glass beads, and titrate with the standard dextrose solution as directed under procedure. Adjust concentration of Fehling's Solution A by dilution or addition of copper sulfate so that the titration requires 20.0 mL of the 0.6% standard dextrose solution.

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5. Methylene Blue Indicator: 1% aqueous solution

PROCEDURE

Weigh accurately an amount of sample such that after hydrolysis and final dilution the solution contains 0.6% total reducing sugars (Note 4). Transfer the sample quantitatively to a 3-necked round bottom distilling flask with 325 mL of purified water. Mount a Centigrade thermometer in one of the necks. Add 75 mL of 4 *N* sulfuric acid and close the center opening with a rubber stopper. Place the flask containing the mixed solution in the electric mantle of the hydrolysis assembly. Place the reflux condenser in the third neck. Heat the solution to the boiling point in about 25 mins. and continue boiling for 150 mins. Remove the flask and cool the hydrolyzate to 25 °C in an ice-water bath.

Transfer the cooled hydrolyzate quantitatively to a 600 mL beaker. Neutralize the solution to about pH 4 with 50% sodium hydroxide and then to pH 5.0 ± 0.1 *N* sodium hydroxide. Transfer the neutralized hydrolyzate quantitatively to a 500 mL volumetric flask, adjust temperature to 25 °C, dilute to volume, mix and filter through Whatman No. 1 filter paper.

Pipet 25.0 mL of standardized mixed Fehling's Solution into a 200 mL Erlenmeyer flask and add a few glass beads. Add the filtered hydrolyzate solution by means of a buret to within 0.5 mL of the anticipated end point (determined by preliminary titration). Immediately place the flask on the wire gauze of the titration assembly, and adjust burner so that the boiling point will be reached in about 2 mins. Bring to boil and boil gently for 2 mins. As boiling continues, add 2 drops of methylene blue indicator, and complete the titration within 1 min. by adding sample solution dropwise or in small increments until the blue color disappears (Note 5).

CALCULATION

$$\%\ \text{Total Sugars (as is, calc. as dextrose)} = \frac{(500\ \text{mL})(0.1200)(100)}{(\text{Sample Titer, mL})(\text{Sample Wt., g})}$$

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NOTES AND PRECAUTIONS

1. The Association of American Feed Control Officials defines starch molasses as "the by-product of the manufacture of dextrose from starch derived from corn or grain sorghums in which the starch is hydrolyzed by use of enzymes and/or acid."

2. This procedure is patterned from that presented by G. T. Peckham and C. E. Engel in their paper entitled, "The Sugar Content of Hydrol (Corn Feeding Molasses)," [*J. Assoc. Offic. Agr. Chem.*, *36*, 457-65 (1953)]. Principal differences are the use of a higher sample concentration during hydrolysis, and use of the Lane and Eynon reducing sugar method for analysis of the hydrolyzate.

3. Fehling's Solution in mixed form is relatively unstable but may be retained up to 1 week if standardization is confirmed before using.

4. Estimate the sample size for starch molasses as follows:

$$\text{Sample Weight} = \frac{(3\,\text{g})(100)}{\%\ \text{Anticipated Total Reducing Sugars}}$$

   The total reducing sugars content of starch molasses after hydrolysis is about 20% greater than the reducing sugars content. Therefore, the optimum sample size is about 85% of that used for reducing sugars analysis, assuming that the final dilution volume is the same for both analyses.

   Concentration should be such that sample titer is near 20 mL but should not exceed limits of 15 and 25 mL. Inter- and intralaboratory precision is improved by adjusting all sample concentrations to provide titers between 19 and 21 mL.

5. When approaching the end point, allow about 5 secs. reaction time between additions of sample solution.

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METHOD HISTORY

Corn Sugar, Total Sugars (F-58), Date of Acceptance 3-20-1972, Revised 2-27-1996.