REDUCING SUGARS

PRINCIPLE

Reducing sugars are most commonly determined by reaction with a stabilized alkaline solution of a copper salt. When reaction conditions, i.e., time, temperature, reagent concentration and composition, are controlled, the amount of copper reduced is proportional to the amount of reducing sugars in the sample analyzed. In the accompanying adaptation of the Schoorl method (Note 1), the reducing sugar concentration expressed as dextrose, is estimated by iodometric determination of the unreduced copper remaining after reaction.

SCOPE

This adaptation of the Schoorl method was designed specifically for analysis of steepwater (Note 2). Only minor modification is required for application to most carbohydrate mixtures containing reducing sugars.

REAGENTS

1. Fehling's Solution (Note 3):

   A. Dissolve 34.65 g of reagent grade crystalline copper sulfate pentahydrate ($CuSO_4 \bullet 5H_2O$) in purified water and dilute to 500 mL.

   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 in purified water and dilute to 500 mL.

2. Phosphotungstic Acid, Crystals: Reagent Grade

3. Potassium Iodide Solution, 30%: Dissolve 150 g of reagent grade potassium iodide (KI) in purified water and dilute to 500 mL. The reagent should be protected from light during storage, and it should be discarded when the presence of free iodine becomes evident.

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4. Sulfuric Acid Solution, 28%: Cautiously pour 85 mL of concentrated sulfuric acid (96% H_2SO_4; sp g 1.84) into 400 mL of purified water while stirring. Cool to room temperature and dilute to 500 mL.

5. Sodium Thiosulfate Solution, 0.1 *N*: Standard

6. Starch Indicator Solution, 1%

PROCEDURE

1. Mix the heavy steepwater sample and weigh accurately about 10 g (Note 4). Transfer to a 200 mL Kohlrausch flask with purified water, add 2 g of phosphotungstic acid crystals, and mix (Note 5). Dilute to 201 mL (Note 6) with purified water, using a drop of amyl alcohol to disperse foam if necessary. Mix, gravity filter through Whatman No. 1 filter paper, collect filtrate in a clean and dry flask.

   Pipet 10.0 mL of Fehling's A solution and then 10.0 mL of Fehling's B solution into a 250 mL Erlenmeyer flask. Add 20.0 mL of sample filtrate and 10 mL of purified water to bring total volume of reaction mixture to 50 mL. Mix contents of flask by swirling.

   Add 2 small glass beads to prevent bumping while boiling, and cover the mouth of the flask with a small glass funnel or glass bulb. Place the flask on a hot plate adjusted to bring the solution to a boil in 3 minutes (Note 7), and continue boiling for exactly 2 minutes (total heating time of 5 minutes). Cool quickly to room temperature in an ice bath or a cold running water bath.

   Add 10 mL of 30% potassium iodide solution and 10 mL of 28% sulfuric acid solution; titrate immediately with standard 0.1 *N* sodium thiosulfate solution. Near the end point add 1 mL of starch indicator solution, and continue titrating carefully while agitating the solution continuously until the blue starch-iodine color is discharged.

   Conduct two blank determinations in identical fashion substituting purified water for the sample.

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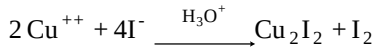
CALCULATION

Subtract sample titer from the average blank titer. Find reducing sugar content (expressed as dextrose) equivalent to the titer difference by reference to the accompanying table (Note 8).

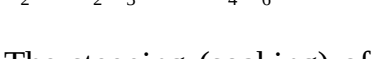
$$\%\ \text{Reducing Sugars (as is) (as Dextrose)} = \frac{\text{mg Dextrose (From Table)} \times 200\ \text{mL} \times 100}{\text{Sample Wt. (g)} \times 20\ \text{mL} \times 1000\ \text{mg/g}}$$

NOTES AND PRECAUTIONS

1. Copper oxidation of carbohydrates is not a simple reaction; end products are mixtures of acids, so equations are not usually written. Iodometric determination of excess copper (II) is summarized:



$$2\,Cu^{++} + 4I^- \xrightarrow{H_3O^+} Cu_2I_2 + I_2$$



$$I_2 + 2\,S_2O_3 = \mapsto S_4O_6 = + 2\,I^-$$

2. The steeping (soaking) of corn in a dilute sulfurous acid solution extracts solubles including carbohydrates, and the latter are largely fermented to lactic acid during the process. The concentrated extract is known as heavy steepwater in the corn wet milling industry, and it is designated Condensed Fermented Corn Extractives by the Association of American Feed Control Officials. The latter organization provides the following definition: "Condensed Fermented Corn Extractives is the product obtained by the partial removal of water from the liquid resulting from steeping corn in a water and sulfur dioxide solution which is allowed to ferment by the action of naturally occurring lactic acid producing microorganisms as practiced in the wet milling of corn."

3. The Fehling's solution recommended here is equivalent to that used in Dextrose Equivalent Methods E-26 and F-22, where careful standardization of the reagent is essential. However, copper concentration in the Schoorl method is not highly critical and adjustment may be unnecessary. Satisfactory results can be obtained with reagents giving blank titers ranging from 27.5 to 29.5 mL of 0.1 *N* sodium thiosulfate solution, and adjustment of the copper sulfate solution (Fehling's A solution) concentration to provide blank titers in this range is recommended.

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4. Use a 100 g sample when analyzing light steepwaters from the process containing about 5% dry substance.

5. Treatment with phosphotungstic acid precipitates proteinaceous materials which interfere in the reducing sugar analysis.

6. Dilution to 201 mL corrects for the precipitate volume, giving a filtrate volume of 200 mL.

7. Alternatively, the flask may be placed on a wire gauze and heated over a Bunsen burner, but control of the heating cycle is more difficult by this technique. Heating on a hot plate with a stepless control is recommended for best precision.

8. Use of the accompanying table for conversion of titer difference to mg of reducing sugar (expressed as dextrose) presumes ability of the analyst to duplicate exactly the conditions under which the table was developed. This approach is quite accurate in most cases but it does entail a risk of error.

9. The risk of error can be avoided by careful duplicate standardization using known quantities of pure dextrose. The standardization plot is slightly curvilinear, passing through the origin.

REFERENCES

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4. Cereal laboratory methods, 3rd Edition, pp 18-19, 1935, American Society of Cereal Chemists

5. Physical and Chemical Methods of Sugar Analysis, Edited by C. A. Browne and F. W. Zerban, 3rd Edition, John Wiley & Sons, Inc., New York, N. Y., 1948

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Schoorl Method: Conversion of Titer Difference to Reducing Sugars

Titer Difference*	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	Reducing Sugars (As Dextrose), mg									
0.0 mL	0.0	0.3	0.7	1.0	1.3	1.6	1.9	2.2	2.5	2.8
1.0	3.2	3.5	3.8	4.1	4.4	4.7	5.0	5.3	5.6	5.9
2.0	6.4	6.6	6.9	7.2	7.5	7.8	8.1	8.5	8.8	9.1
3.0	9.4	9.8	10.1	10.4	10.7	11.0	11.4	11.7	12.0	12.3
4.0	12.6	13.0	13.3	13.6	14.0	14.3	14.6	15.0	15.3	15.6
5.0	15.9	16.3	16.6	16.9	17.2	17.6	17.9	18.2	18.5	18.9
6.0	19.2	19.5	19.8	20.1	20.5	20.8	21.1	21.4	21.8	22.1
7.0	22.4	22.7	23.0	23.3	23.7	24.0	24.3	24.6	24.9	25.2
8.0	25.6	25.9	26.2	26.6	26.9	27.3	27.6	28.0	28.3	28.6
9.0	28.9	29.3	29.6	30.0	30.3	30.6	31.0	31.3	31.6	31.9
10.0	32.3	32.7	33.0	33.3	33.7	34.0	34.3	34.6	35.0	35.3
11.0	35.7	36.0	36.3	36.7	37.0	37.3	37.6	38.0	38.3	38.7
12.0	39.0	39.3	39.6	40.0	40.3	40.6	41.0	41.3	41.7	42.0
13.0	42.4	42.8	43.1	43.4	43.7	44.1	44.4	44.8	45.2	45.5
14.0	45.8	46.2	46.5	46.9	47.2	47.6	47.9	48.3	48.6	48.9
15.0	49.3	49.6	49.9	50.3	50.7	51.1	51.4	51.7	52.1	52.4
16.0	52.8	53.2	53.5	53.9	54.2	54.5	54.9	55.3	55.6	56.0
17.0	56.3	56.7	57.0	57.3	57.7	58.1	58.4	58.8	59.1	59.5
18.0	59.8	60.1	60.5	60.9	61.2	61.5	61.9	62.3	62.6	63.0
19.0	63.3	63.6	64.0	64.3	64.7	65.0	65.4	65.8	66.1	66.5
20.0	66.9	67.2	67.6	68.0	68.4	68.8	69.1	69.5	69.9	70.3
21.0	70.7	71.1	71.5	71.9	72.2	72.6	73.0	73.4	73.7	74.1
22.0	74.5	74.9	75.3	75.7	76.1	76.5	76.9	77.3	77.7	78.1
23.0	78.5	78.9	79.3	79.7	80.1	80.5	80.9	81.3	81.7	82.1
24.0	82.6	83.0	83.4	83.8	84.2	84.6	85.0	85.4	85.8	86.2
25.0	86.6	87.0	87.4	87.8	88.2	88.6	89.0	89.4	89.8	90.2
26.0	90.7	91.1	91.5	91.9	92.3	92.7	93.1	93.5	93.9	94.3
27.0	94.8									

*mL of 0.1000 *N* sodium thiosulfate solution.