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Chapter

Glucose Determination by Enzyme Analysis

Charles E. Carpenter (✉) • Robert E. Ward

Department of Nutrition, Dietetics and Food Sciences, Utah State University,
Logan, UT, USA

e-mail: chuck.carpenter@usu.edu; robert.ward@usu.edu

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24.1 INTRODUCTION

24.1.1 Background

Enzyme analysis is used for many purposes in food science and technology. Enzyme activity is used to indicate adequate processing, to assess enzyme preparations, and to measure constituents of foods that are enzyme substrates. In this experiment, the glucose content of corn syrup solids is determined using the enzymes, glucose oxidase and peroxidase. Glucose oxidase catalyzes the oxidation of glucose to form hydrogen peroxide (H_2O_2), which then reacts with a dye in the presence of peroxidase to give a stable-colored product.

As described, this experiment uses individual, commercially available reagents, but enzyme test kits that include all the reagents to quantitate glucose also are available as a package. Enzyme test kits also are available to quantitate various other components of foods. Companies that sell enzyme test kits usually provide detailed instructions for the use of these kits, including information about the following: (1) principle of the assay, (2) contents of the test kit, (3) preparation of solutions, (4) stability of solutions, (5) procedure to follow, (6) calculations, and (7) further instructions regarding dilutions and recommendations for specific food samples.

24.1.2 Reading Assignment

BeMiller, J.N. 2017. Carbohydrate analysis. Ch. 19, in *Food Analysis*, 5th ed. S.S. Nielsen (Ed.), Springer, New York.

Reyes-De-Coreuera, J.I., and Powers, J.R. 2017. Application of enzymes in food analysis. Ch. 25, in *Food Analysis*, 5th ed. S.S. Nielsen (Ed.), Springer, New York.

24.1.3 Objective

Determine the glucose content of food products using the enzymes, glucose oxidase and peroxidase.

24.1.4 Principle of Method

Glucose is oxidized by glucose oxidase to form hydrogen peroxide, which then reacts with a dye in the presence of peroxidase to give a stable-colored product that can be quantitated spectrophotometrically (coupled reaction).

24.1.5 Chemicals

	CAS No.	Hazards
Acetic acid (Sigma A6283)	64-19-7	Corrosive
o-Dianisidine 2HCl (Sigma D3252)	20325-40-0	Tumor causing, carcinogenic
D-Glucose (Sigma G3285)	50-99-7	

Glucose oxidase (Sigma G6641)	9001-37-0	
Horseradish peroxidase (Sigma P6782)	9003-99-0	
Sodium acetate (Sigma S2889)	127-09-3	
Sulfuric acid (Aldrich 320501)	7664-93-9	Corrosive

24.1.6 Reagents

(**It is recommended that these solutions be prepared by the laboratory assistant before class.)

- Acetate buffer**, 0.1 M, pH 5.5
Dissolve 8 g sodium acetate in ca. 800 mL water in a 1-L beaker. Adjust pH to 5.5 using 1 M HCl. Dilute to 1 L in a volumetric flask.
- Glucose test solution**
In a 100-mL volumetric flask, dissolve 20 mg glucose oxidase (~300–1000 units), 40 mg horseradish peroxidase, and 40 mg o-dianisidine 2HCl in the 0.1 M acetate buffer. Dilute to volume with the acetate buffer and filter as necessary.
- Glucose standard solution, 1 mg/mL
Use commercial D-glucose solution (e.g., Sigma).
- Sulfuric acid, diluted** (1 part H_2SO_4 +3 parts water)
In a 500-mL beaker in the hood, add 150 mL water and then add 50 mL H_2SO_4 . This will generate a lot of heat.

24.1.7 Hazards, Precautions, and Waste Disposal

Concentrated sulfuric acid is extremely corrosive; avoid contact with skin and clothes and breathing vapors. Acetic acid is corrosive and flammable. Wear safety glasses at all times and corrosive-resistant gloves. Otherwise, adhere to normal laboratory safety procedures. The o-dianisidine 2HCl must be disposed of as hazardous waste. Other waste likely may be put down the drain using a water rinse, but follow good laboratory practices outlined by environmental health and safety protocols at your institution.

24.1.8 Supplies

- Beaker, 1 L
- Corn syrup solids (or high fructose corn syrup), 0.5 g
- 5 Spatulas
- 14 Test tubes, 18×150 mm, heavy walled to keep from floating in water bath
- Test-tube rack

- 2 Volumetric flasks, 100 mL
- Volumetric flask, 250 mL
- Volumetric pipette, 10 mL
- 2 volumetric flasks, 1 L
- Weighing paper

24.1.9 Equipment

- Analytical balance
- Mechanical, adjustable volume pipettors, 200, 1000, and 5000 μL , with tips
- pH meter
- Spectrophotometer
- Water bath, 30 $^{\circ}\text{C}$

24.2 PROCEDURE

(Instructions are given for analysis in duplicate.)

1. Prepare dilutions for standard curve. Use the adjustable pipettors to deliver aliquots of glucose standard solution (1 mg/mL) and deionized distilled (dd) water as indicated in the table below into clean test tubes. These dilutions will be used to create a standard curve of 0–0.2 mg glucose/mL.

	mg glucose/mL				
	0	0.05	0.10	0.15	0.20
mL glucose std. solution	0	0.150	0.300	0.450	0.600
mL dd water	3.000	2.850	2.700	2.550	2.400

2. Prepare sample solution and dilutions. Accurately weigh ca. 0.50 g corn syrup solids and dilute with water to volume in a 250-mL volumetric flask (Sample A). Using volumetric pipettes and flasks, dilute 10.00 mL of Sample A to 100 mL with water (Sample B). These sample dilutions will let you determine glucose concentrations in samples containing 1–100% glucose.
3. Add 1.000 mL of water to each of 14 test tubes. In duplicate, add 1.000 mL of the individual standard and sample dilutions to the test tubes.
4. Put all tubes in the water bath at 30 $^{\circ}\text{C}$ for 5 min. Add 1.000 mL glucose test solution to each tube at 30 s intervals.
5. After exactly 30 min, stop the reactions by adding 10 mL of the diluted H_2SO_4 . Cool to room temp.
6. Zero spectrophotometer with water in the reference position using a double beam spectrophotometer. Take two readings (repeated measures, msmt) using separate aliquots from each tube.

24.3 DATA AND CALCULATIONS

Weight of original sample: _____ g

Absorbance of standard solutions:

Tube	Msmt	(mg glucose/mL)				
		0	0.05	0.10	0.15	0.20
1	1					
	2					
2	1					
	2					

Average absorbance:

Absorbance of samples:

Tube	Msmt	Sample A	Sample B
1	1		
	2		
2	1		
	2		

Average absorbance:

Calculation of glucose concentration in sample:

1. Plot absorbance of standards on the y -axis versus mg glucose/mL on the x -axis.
2. Calculate the concentration of glucose for the sample dilution A or B that had an absorbance within the working range of the standard curve: $(Abs - y\text{-intercept})/\text{slope} = \text{mg glucose/mL}$.
3. Calculate the glucose concentration in the original sample, as a percentage.

Example calculations:

Original sample of 0.512 g

Average measured absorbance sample dilution B: 0.200

Calculation from standard curve: $0.200 - 0.003 / (2.98 \text{ mL/mg glucose}) = 0.066 \text{ mg glucose/mL B}$

$$\begin{aligned}
 C_{\text{sample}} &= (0.066 \text{ mg glucose/mL B}) \\
 &\quad \times (100 \text{ mL B} / 10 \text{ mL A}) \\
 &\quad \times (250 \text{ mL A} / 512 \text{ mg sample}) \\
 &= 0.323 \text{ mg glucose/mg sample} \\
 &= 32.3\% \text{ glucose}
 \end{aligned}$$

24.4 QUESTIONS

1. Explain why this experiment is said to involve a coupled reaction. Write in words the equations for the reactions. What conditions must be in place to ensure accurate results for such a coupled reaction?
2. How do the results obtained compare to specifications for the commercial product analyzed?

RESOURCE MATERIALS

BeMiller JN (2017) Carbohydrate analysis. Ch. 19. In: Nielsen

SS (ed) Food analysis, 5th edn. Springer, New York
Reyes-De-Coreuera JI, and Powers JR (2017) Application of
enzymes in food analysis. Ch. 26. In: Nielsen SS (ed) Food
analysis, 5rd edn. Springer, New York