

The Enzymatic Hydrolysis of Sucrose



Introduction

Using simple laboratory techniques, demonstrate the production of the enzyme invertase (sucrase) by yeast. The demonstration can lead to a discussion of the role invertase plays in yeast metabolism.

Concepts

- Enzymes
- Hydrolysis

Materials (for each demonstration)

| | |
|--|------------------------------------|
| Benedict's qualitative solution, 60 mL | Filter paper, creped or fast speed |
| Sucrose, 5 g | Filtering funnel |
| Yeast, active dry (baker's), 7 g package | Graduated cylinder, 10-mL |
| Water, distilled or deionized | Hot plate |
| Beaker, Pyrex®, 600- or 1000-mL | Test tube rack |
| Beral-type pipets, 3 | Test tubes, 18 × 150 mm, 6 |

Safety Precautions

Benedict's qualitative solution is a skin and eye irritant. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Use insulated gloves or test tube clamps when handling the heated test tubes during the Benedict's procedure. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

Prepare a yeast filtrate solution by mixing one package of active dry yeast with 80 mL of distilled or deionized water. Let stand for approximately 20 minutes with occasional stirring. Filter the resulting suspension and save the filtrate solution—this is the crude invertase extract. Vacuum filtration or centrifugation are time-saving options to gravity filtration if equipment is available. Refrigerate the invertase extract if held overnight. Prepare a 5% sucrose solution by dissolving 5 grams of sucrose (highest purity available) in 100 mL of distilled or deionized water. Prepare the sucrose solution shortly before use.

Procedure

1. Prepare a boiling water bath using a Pyrex beaker and a hot plate or Bunsen burner setup (used in step 5).
2. Place three test tubes (labeled A1, A2, A3) in a test tube rack. Into tube A1, place 20 mL of 5% sucrose solution. Into tube A2, place 20 mL of 5% sucrose solution and 4 mL of invertase extract. Into tube A3, place 20 mL distilled or deionized water and 4 mL of invertase extract. Stopper and invert each tube to mix.
3. Incubate the tubes for 35 minutes in warm, 30 to 35 °C, water. Use warm tap water (check temperature) in a beaker or Styrofoam® cup.
4. Place three more test tubes (labeled B1, B2, B3) in a test tube rack. Place 20 mL of Benedict's qualitative solution into each. Transfer 32 drops of the contents of A1 into B1, 32 drops of A2 into B2, and 32 drops of A3 into B3.
5. Place tubes B1, B2, and B3 into a boiling (or near boiling) water bath. After three or four minutes, note whatever changes are evident.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. The resulting mixtures can all be rinsed down the drain according to Flinn Suggested Disposal Method #26b.

Tips

- A glucose “standard” tube can be added at step 4 for comparison. Label this tube B4 and into it place 20 mL of Benedict's qualitative solution and 32 drops of a 5% glucose (dextrose) solution. Place tube B4 in the boiling water

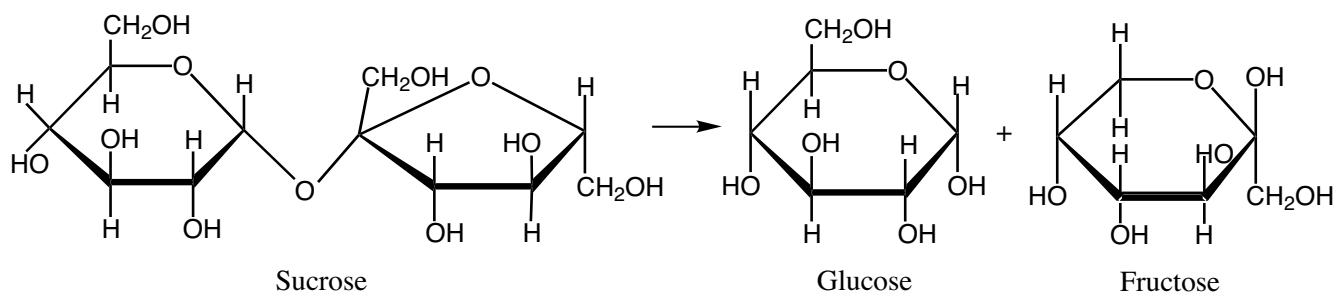
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bath with the others in step 5.

- Standard proportions for the Benedict's (qualitative) test are 5 mL of Benedict's solution and 8 drops of the sugar/test solution. The volumes in this lab are scaled up for visibility.

Discussion

The enzyme invertase (sucrase, saccharase) catalyzes the hydrolysis of sucrose (a disaccharide) to invert sugar. *Invert sugar* is a 50/50 mixture of glucose and fructose—both monosaccharides. Yeast cannot directly metabolize (ferment) sucrose. In order to utilize sucrose as an energy source, the yeast must first convert it to fermentable monosaccharides—glucose and fructose.



The Benedict's solution is used to show whether or not the conversion of sucrose to invert sugar has occurred. Benedict's qualitative solution is a test reagent that reacts positively with (simple) reducing sugars. All monosaccharides and most disaccharides are reducing sugars—that is, they possess a free, or potentially-free carbonyl group ($C = O$). Sucrose is an exception in that it is not a reducing sugar. A positive Benedict's test is evidenced by the formation of a brownish-red cuprous oxide precipitate. Glucose and fructose both test positively with Benedict's solution—sucrose does not.

Tubes A1/B1 show that sucrose alone will not reduce Benedict's solution. Tubes A2/B2 show, quite distinctly, the ability to reduce Benedict's solution—from which we can infer the presence of reducing sugars (in this case, glucose and fructose). Tubes A3/B3 serve as an experimental control—to show that the positive reaction is not due to the invertase extract itself. If a glucose standard tube (B4) is incorporated A1/B1, it can be compared directly to tube B2 following the Benedict's test.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12

- Evidence, models, and explanation
- Constancy, change, and measurement

Content Standards: Grades 5–8

- Content Standard B: Physical Science, properties and changes of properties in matter

Content Standards: Grades 9–12

- Content Standard B: Physical Science, structure of atoms, structure and properties of matter, chemical reactions

Reference

Morholt, E.; Brandwein, P. F.; *A Sourcebook for the Biological Sciences*, 3rd ed.; Harcourt Brace Jovanovich: Orlando, FL 1986; pp 247–248.

Materials for *The Enzymatic Hydrolysis of Sucrose* are available from Flinn Scientific, Inc.

| Catalog No. | Description |
|-------------|---|
| B0015 | Benedict's Qualitative Solution, 500 mL |
| B0016 | Benedict's Qualitative Solution, 1 L |
| S0134 | Sucrose, reagent grade, 500 g |
| Y0008 | Yeast, baker's, three 7-g packets |
| AP3113 | Filter Paper, 11 cm |

Consult the [Flinn Scientific website](#) for current prices.