

Lactase Enzyme Lab

This lab will examine the specificity of an enzyme (lactase) to a specific substrate (lactose). Students will observe what happens when the enzyme is denatured.

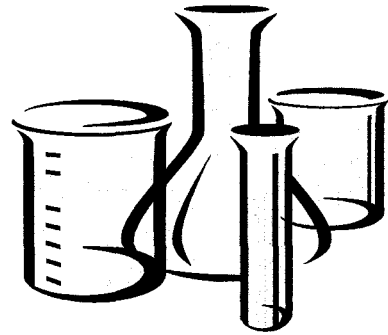
Introduction

Lactose, the sugar found in milk, is a disaccharide composed of glucose and galactose (both six-sided sugars). Sucrose, ordinary table sugar, is also a disaccharide composed of fructose and glucose. Glucose is a six-sided sugar and fructose is a five-sided sugar.

Lactase is an enzyme that breaks lactose down into galactose and glucose. Lactase can be purchased in pill form for people who are lactose intolerant. These people lack the enzyme, lactase, and cannot break down the sugar lactose into its component parts.

Although lactose is similar to sucrose, lactase will break down only lactose because of the shape of the sugar.

In this lab, you will see lactase break lactose down into galactose and glucose. You will also observe what happens when lactase is denatured (the shape of the active site is changed due to heating).



Materials & Methods

- Four prepared solutions: see *Solution Preparation* below.
- Five 50mL beakers: to hold the different solution mixtures (A-E).
- Safety goggles
- Hot plate with a Pyrex beaker and test tube for denaturing the enzyme.
- Test tube rack: hold the cooling test tube.
- Four 10mL graduated cylinders or droppers: measuring solution and enzyme amounts. *Note: use a different graduated cylinder/dropper for each solution! Prevent contamination!*
- Marking pencil: mark the test tubes so that confusion does not occur.
- Glucose test strips
- Forceps: to hold and dip the glucose test squares. *Do not touch strips with your fingers.*

Solution Preparation

1. Enzyme solution: Add $\frac{1}{2}$ lactase tablet to 100mL of water. Stir until the tablet has dissolved.
2. Skim milk: this solution contains the lactose.
3. Sucrose solution: Add five grams of sugar to 100mL of water. Stir until the sugar has dissolved.
4. Denatured enzyme solution:
 - Place 20mL of enzyme solution into a Pyrex test tube.
 - Add 200mL of water to a 400mL Pyrex beaker.
 - Place the test tube in the beaker (place the test tube so it rests on the side of the beaker).
 - Place the beaker and test tube on the hot plate and boil for 25 minutes.
 - Let the solution cool to room temperature.

Sodek
Lab Procedures

- Label the 50mL beakers with the following labels:
 - Beaker with skim milk and enzyme solution.
 - Beaker with skim milk and water.
 - Beaker with skim milk and denatured enzyme solution.
 - Beaker with sucrose solution and enzyme solution.
 - Beaker with sucrose solution and water.
- In beaker A add 2mL of skim milk and one milliliter of enzyme solution.
- Time for two minutes and test for glucose with the glucose test tape. Record this data in the table. If there was glucose present mark a '+' in the table. If glucose was absent, mark a '-' in the table.
- In beaker B add 2mL of skim milk and one milliliter of water.
- Repeat step 3.
- In beaker C add 2mL of skim milk and 1mL of denatured enzyme solution.
- Repeat step 3.
- In beaker D add 2mL of the sucrose solution and 1mL of enzyme solution.
- Repeat step 3.
- In beaker E add 2mL of the sucrose solution and 1mL of water.
- Repeat step 3.

Results:

Record if the glucose test is positive or negative for each of the test tubes.

Type of Solution	Positive or Negative Glucose Result
Beaker A: milk and enzyme solution	
Beaker B: milk and water	
Beaker C: milk and denatured enzyme solution	
Beaker D: sucrose solution and enzyme solution	
Beaker E: sucrose solution and water	

Conclusion and questions

- Diagram and describe the lactose and lactase reaction.
- Why did the enzyme react to lactose but not to sucrose?
- What happened when the enzyme was boiled?
- Another way to affect the enzyme is by lowering the pH of the solution. However, lactase is supposed to be able to work in the stomach. Would lowering the pH of the enzyme solution affect the enzyme? Why or why not?
- What type of reaction is this? Dehydration or hydrolysis?
- Explain five sources of error for this lab.