Pectinase Ate My Fruit

Enzymes Used in the Food Industry

Introduction

Many naturally occurring enzymes are used in the food and beverage industry to improve food processing, juice clarity, and taste. Pectinase, an enzyme that breaks down pectin in fruits, is commercially important in making fruit juices. Let's examine the activity of pectinase.

Concepts

- Enzymes
- Biochemical processes

Background

Pectins are a family of complex polysaccharides that are a major component of primary cell walls in plants. Specifically, pectins are a major component of the middle lamella, the layer between neighboring cells. Pectins determine the pore size of cell walls and are involved in defense mechanisms against wounding, stress and infections by plant pathogens, and play a role in water retention.

Many bacteria and fungi that use plants for their food source contain high levels of pectinase, a naturally occurring enzyme that breaks down pectin. Pectinase is easily harvested from sources such as Aspergillus niger, a mold that secretes pectinase for extra-cellular digestion of the fruit.

Pectinase has many commercial applications, particularly in the fruit juice industry. Fruits with tough peels, such as oranges and peaches, are soaked in pectinase solutions prior to juice extraction. The enzyme digests the pectin in the peel, and the peel can then be easily removed and the fruit further processed. Pectinase can also be used to clarify fruit juices such as lemon juice and black currant juice, because it digests the insoluble complex pectin carbohydrates in the juice. Pectinase is used in the manufacturing of apple juice, to increase yield. Apples are incubated in pectinase solutions and, depending on the conditions, the pressed fruit can produce up to 20% more juice than in an ordinary process.

Materials

Acetic acid, CH_3CO_2H, 0.1 M, 36 mL
Pectinase, 5 g
Sodium acetate, CH_3CO_2Na, 0.1 M, 64 mL
Apples
Balance, 0.1-g precision
Beakers, 100-mL, 2
Beakers, 250-mL, 2
Cheesecloth, 8″ × 8″, 2
Funnels, short-stemmed, 2
Glass stirring rods, 2
Graduated cylinders, 10-mL, 2
Graduated cylinders, 25-mL, 2
Knife, food chopper, or apple slicer
Plastic wrap or Parafilm®, 6″, 2
Water bath, 45 °C
Wax pencil or marker
Weighing dishes, 2

Safety Precautions

Sodium acetate causes mild skin and eye irritation. All food-grade items that have been brought into the lab are considered laboratory chemicals and are for lab use only. Do not taste or ingest any materials in the lab. Avoid contact of all solutions with eyes and skin. Wear chemical splash goggles and chemical-resistant gloves. Wash hands thoroughly with soap and water before leaving the laboratory.

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Pre-Lab Preparation

1. Prepare a 0.1 M sodium acetate ($CH_3CO_2Na•3H_2O$) solution by dissolving 13.6 g of sodium acetate in 100 mL of distilled or deionized water.
2. Prepare 100 mL of an acetate buffer, pH 5 (enough for 10 groups of students)
   a. Use a wax pencil or marker to label a 100-mL beaker "acetate buffer."
   b. Pour 36 mL of 0.1 M acetic acid into the labeled beaker.
   c. Add 64 mL 0.1 M sodium acetate into the 100-mL beaker and mix.
3. Prepare 50 mL of a 10% pectinase solution (enough for 10 groups of students)
   a. Use a wax pencil or marker to label a 100-mL beaker "10% pectinase."
   b. Use a graduated cylinder to measure 50 mL of the acetate buffer into the labeled beaker.
   c. Using a balance, measure 5 g pectinase into a weighing dish.
   d. Add the pectinase to the acetate buffer in the labeled beaker.
   e. Use a glass stirring rod to mix.
4. Cut the cheesecloth into 8″ × 8″ squares.
5. Set up a 45 °C water bath.

Procedure

1. Use a wax pencil or marker to label a 250-mL beaker and a 25-mL graduated cylinder "pectinase." Label the second 250 mL beaker and 25-mL graduated cylinder "buffer."
2. Place a funnel into each 25-mL graduated cylinder.
3. Fold one 8″ × 8″ square piece of cheesecloth twice to form a four-layer 4″ × 4″ square. Repeat for the second piece of cheesecloth.
4. Place one four-layer piece of cheesecloth into each funnel.
5. Use a sharp knife, food chopper or apple slicer to cut an apple into 0.5-cm cubes.
6. Using a balance, measure 50 g of apple pieces into a weighing dish.
7. Add 50 g of apple pieces to one of the two labeled 250-mL beakers. Use a glass stirring rod to break apart the apple pieces.
8. Repeat steps 6 and 7, adding the apple pieces into the second labeled 250-mL beaker.
9. Use a 10-mL graduated cylinder to measure 5 mL of acetate buffer. Pour the buffer into the "buffer" labeled beaker.
10. Use a clean, 10-mL graduated cylinder to measure 5 mL of 10% pectinase solution. Pour the 10% pectinase solution into the "pectinase" labeled beaker.
11. Cover both beakers with plastic wrap or Parafilm® and incubate the apple mixture in the 45 °C water bath for 20 minutes.
12. Pour the contents of the "buffer" beaker into the cheesecloth-lined funnel in the "buffer" labeled 25-mL graduated cylinder. Repeat using the "pectinase" beaker and "pectinase" graduated cylinder.
13. Measure the amount of juice filtered into each graduated cylinder every 5 minutes for 15 minutes. Stir the apple pieces occasionally with a glass stirring rod to release juice that has become caught among the apple pieces.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures, and review all federal, state and local regulations that may apply, before proceeding. All of the leftover solutions may be disposed of according to Flinn Suggested Disposal Method #26b. The apple solid may be disposed of according to Flinn Suggested Disposal Method #26a, solid waste disposal in a landfill.

NGSS Alignment

This laboratory activity relates to the following Next Generation Science Standards (2013):

Disciplinary Core Ideas: Middle School
MS-PS1 Matter and its Interactions
PS1.B: Chemical Reactions
Disciplinary Core Ideas: High School
HS-PS1 Matter and its Interactions
PS1.B: Chemical Reactions
HS-LS1 From Molecules to Organisms: Structures and Processes
LS1.A: Structure and Function

Science and Engineering Practices
Planning and carrying out investigations
Analyzing and interpreting data

Crosscutting Concepts
Cause and effect
Structure and function

Tips

- Any variety of apples should work. The procedure was tested using Red Delicious. Some sources cite problems with Golden Delicious.
- An apple slicer, mandolin or manual food chopper will quickly dice the apples.
- Try using apple sauce or other fruits instead of apples.
- During testing, no juice was collected from the buffer sample, while a total of 6.5 mL was collected from the pectinase-treated sample.
- Do not allow students to consume apple pieces or juice. Remind them that all food products become laboratory chemicals once they enter the lab.
- All enzymes have optimal temperature and pH ranges—for pectinase the optimal temperature is 40–50 °C and the optimal pH is about 5. At 60 °C, heat begins to alter the structure of the enzyme, denaturing it so that it can no longer break down pectin molecules. If the pH of the enzyme's environment is too low, H^+ ions will protonate with basic amino acid side chains on the enzymes and possibly alter their activity. If the pH is too high, OH^- ions will deprotonate acidic side chains on the enzyme that may be needed for enzyme activity.

Materials for *Pectinase Ate My Fruit* are available from Flinn Scientific, Inc

Catalog No.	Description
P0285	Pectinase, 5 g
A0096	Acetic acid, 0.1 M, 1 L
S0036	Sodium acetate, 100 g
FB0570	Cheesecloth, 4 sq, yds.

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.