

TEACHER INFORMATION SHEET

TESTING FOR CATALASE ACTIVITY

Taken from IFT Experiments in Food Science Series

The chemical reactions that occur in living cells are complex and highly controlled. Certain chemicals accelerate reactions without being changed themselves. These outside agents are called *catalysts*, and the rate acceleration caused by such substances is called *catalysis*.

Practically all biological reactions involve the use of specialized catalysts called *enzymes*. Enzymes are produced naturally in plant, animal, and microbial cells, and thousands of different enzymes can be found in any cell. Because each enzyme has a very specific function in the cell and is not used up during the reaction, only low concentrations need to be present to achieve a desired effect.

Enzymes have been of major importance since ancient times, when they were first used in the production of wines, cheeses, and other foods and beverages. Since the mechanisms of enzyme action were not yet known, the control of these enzymatic reactions was based exclusively on practical experience. However, recent understanding of the properties, production, and application of enzymes has changed enzymatic processing from an art to a science.

CLASSES OF ENZYMES

Enzymes can be classified by the type of substance they affect. Proteases (P) react with proteins, lipases (L) with lipids (fats), and carbohydrases (C) with carbohydrates (sugars and starches). Other enzymes (O) react with a variety of other substrates (Visual Master 1).

SOME DESIRABLE CHANGES PRODUCED BY ENZYMES

- Formation of cheese curd by rennin (P)
- Meat tenderizing by papain, bromelin, microbial protease (P)
- Contact-lens cleaning by microbial protease (P)
- Protein digestion by pepsin, trypsin (P)
- Development of flavor in cheeses by lipase, esterase (L)
- Increased loaf volume in bread by amylase (C)
- Prevention of staling in bread by amylase (C)
- Fruit ripening by polygalacturonase (C)
- Removal of fruit juice from pulp by pectinase (C)
- Production of soft center in chocolate covered cherries by invertase (C)
- Production of beer by amylase, glucoamylase, protease (C,P)
- Production of light beer by amylase (C)
- Debittering of fruit juices by naringinase (O)

- Production of low- and no-lactose dairy products (for persons with lactose intolerance) by lactase (C)
- Starch digestion by salivary amylase (C)
- Clarifying of juice drinks by amylase (C)
- Prevention of sloughing in canned green beans by pectin methylesterase (C)

SOME UNDESIRABLE CHANGES PRODUCED BY ENZYMES

- Rotting of foods by protease (P)
- Development of rancidity in fats by microbial lipase (L)
- Over-ripening of fruits by polygalacturonase (C)
- Softening of unblanched vegetables by peroxidase (O)
- Development of off-flavors and off-odors in unblanched vegetables by catalase, lipoxygenase (O)
- Browning of certain fruit and vegetable slices by polyphenolase (O)

ACTION OF ENZYMES



In an enzyme-catalyzed reaction, the enzyme joins with the substrate (a reactant in a chemical reaction) and helps transform the substrate into the product. For example, the enzyme amylase joins with a starch molecule and helps break down the starch into its component sugars. The enzyme is a catalyst, which means that it participates in the reaction but is not used up during the reaction. Enzymes are complex proteins, and each enzyme has a specific structure designed to work with a particular reaction. Like all proteins, enzymes contain chains of amino acids, which are arranged in a specific three-dimensional pattern held together by a variety of chemical forces. A small portion of the enzyme, known as the active site, binds to the substrate.

Figure 1 (Visual Master 2) illustrates the two simple models which explain how the active site of an enzyme can bind specifically to a particular substrate. The first is the *Lock-and-Key model*. This model assumes that the substrate fits into the active site much like a key fits into a lock or the pieces of a jigsaw puzzle interlock. The second is the *Induced-Fit model*. This model assumes that the active site of the enzyme is modified by interaction with the substrate so that the enzyme can mold around the substrate for an appropriate fit.

FACTORS AFFECTING ENZYME ACTIVITY

By following the amount of product formed in a specified period of time, the activity or effectiveness of the enzyme can be assessed quantitatively. As shown in Figure 2 (Visual Master 2), the amount of product formed increases if the concentration of substrate is increased, until there is an excess level of substrate present.

Since enzymes are complex proteins, any factors that affect protein structure will also affect enzyme activity. These factors include pH, temperature, and the presence of other compounds. An enzyme-catalyzed reaction can be speeded up, slowed down, or even stopped by changing any of these factors. In general, enzyme activity doubles for every 10°C rise in temperature until the enzyme is inactivated, at which point the activity drops sharply (Figure 3, Visual Master 3). The “temperature optimum” will vary depending on the amount of time the enzyme is allowed to remain at a specific temperature. What would you suppose is the optimum temperature for enzymes in the human body?

Temperature is often used to control enzyme activity. Vegetables, whole or sliced, are blanched prior to freezing to inactivate catalase, peroxidase, and lipoxygenase enzymes responsible for softening or the development of off-flavors and off-odors. Fruits and vegetables are refrigerated to decrease the rate of browning catalyzed by the enzyme polyphenolase.

Each enzyme has an optimum pH range for activity and also for stability (Figure 4, Visual Master 3). For example, an enzyme that catalyzes the breakdown of protein in the acidic environment of your stomach might have an optimum pH of 2. In the basic environment of the small intestine, the optimum pH of the enzymes will be about 8. Would you expect an enzyme that is used with a citrus fruit to have an acidic or basic pH optimum?

Enzymes are named after their substrates or their type of reaction. Nearly all enzymes have names that end in *-ase*. The prefix is either from the type of reaction or from the name of the substrate. For example, a hydrolase catalyzes hydrolysis reactions, and lipase and chlorophyllase catalyze the hydrolysis of lipids and chlorophyll, respectively.

TEACHER ACTIVITY GUIDE

TESTING FOR CATALASE ACTIVITY

EXPECTED OUTCOMES

This experiment will illustrate that as a vegetable naturally high in the catalase enzyme (e.g., a potato) is heated, there is less and less active catalase. Presence of this enzyme can be monitored by its ability to convert hydrogen peroxide into oxygen and water. As oxygen is produced, a paper disk in the test tube will rise. As the catalase is inactivated, oxygen production decreases, and the ascent of the disk in the test tube will slow accordingly.

ACTIVITY OBJECTIVE

Since all enzymes are proteins and have an optimum activity environment, they are subject to destruction as heat is applied. Control of undesirable enzymatic activity can be achieved in processed fruits and vegetables by blanching prior to freezing. This experiment will illustrate to the student that:

1. Enzymes are naturally present in plant tissue.
2. Enzymes can be controlled by altering their environment, e.g., by adding heat.
3. Catalase activity can be simply detected by monitoring its ability to decompose hydrogen peroxide into oxygen and water.

ACTIVITY LENGTH

Approximately 1 hour

SCIENTIFIC PRINCIPLES

Enzymes are produced naturally in plant, animal, and microbial cells, and thousands of different enzymes can be found in any cell. Because each enzyme has a very specific function in the cell and is not used up during the reaction, only low concentrations need to be present to achieve a desired effect.

Two of the more heat-resistant and widely distributed enzymes in plant tissues are *peroxidase* and *catalase*. Although peroxidase is more heat stable than catalase, the activity of both enzymes has been used to monitor the adequacy of blanching procedures. If both are inactivated, it then can be assumed that other significant flavor- and texture-altering enzymes, such as lipoxy-genase, also are inactivated.

The heating time necessary to destroy catalase or peroxidase depends on the type and size of the fruit or vegetable, the method of heating, the temperature of the heating

medium, and the pH of the blanch water. If the catalase is not inactivated prior to freezing a product, enzymatic deterioration will continue and undesirable “hay-like” flavors and odors will be noted in the final product.

VOCABULARY

- *Blanching* – a mild heat treatment given to vegetables to inactivate or activate enzymes prior to freezing. Blanching temperature (and time) varies with different products and can range from 50 to 100°C.
- *Catalase* – an enzyme naturally present in plant material. If not inactivated, it will produce off-flavors and off-odors in the frozen product.
- *Catalyst* – a substance that is able, in relatively low concentration, to accelerate the rate of a chemical reaction without itself being permanently changed.
- *Enzyme* – any of various protein-like substances in plant and animal cells that act as organic catalysts in initiating or speeding up chemical reactions.
- *Substrate* – a reactant in a chemical reaction.

MATERIALS REQUIRED

- Small red-skinned potatoes
- Water
- Blender
- Coffee filters
- Beaker (250 mL) or measuring cup
- Plastic medicine cups (30 mL), 7 per group
- Hot plate
- Thermometers, 3-4
- Ice bath
- Test tubes (13 mm x 100 mm), 7 per group
- Hydrogen peroxide solution (3%)
- Dropping pipettes for dispensing the hydrogen peroxide
- Small glass rod, 1 per group
- Wax pencil or marker
- Metric ruler
- Paper punch
- Clock or other timing device

INSTRUCTIONAL STRATEGIES AND PROCEDURES

PART I – PREPARATION AND HEAT TREATMENT OF POTATO MIXTURE

1. Divide the class (students) into groups.

2. Give each group 7 plastic medicine cups. Have each group number its cups from 1 to 7.
3. Place 250 mL (1 cup) of water into the blender. Cut up a potato and add it to the water. Blend until smooth. Filter the mixture through a clean coffee filter into a 250 mL beaker. Take the temperature of the contents of the beaker and have the students record it as “initial temperature” on the data table. Pour approximately 5 mL of the filtered potato mixture into each group’s cup #1. Tell them to set this aside until later.
4. Place the beaker on a hot plate and begin heating. When the temperature of the mixture reaches 30°C, transfer approximately 25 mL to one group’s cup 2. Have one student from the group place the cup in an ice bath. Instruct the student to stir the mixture until the contents reach the “initial temperature” once again. The cup should then be removed from the ice bath and set aside until later.
5. Continue heating the mixture on the hot plate, removing additional samples at 35, 40, 45, 50, and 55°C. The samples should be placed in cups 3-7, respectively, and cooled as described in step 3. The cooled mixtures should be divided up so that each group has 7 cups of material to test for Part II. Have each group follow the steps outlined in Part II.

PART II – TESTING FOR CATALASE ACTIVITY

1. Number each groups of test tubes from 1 to 7. Place a mark 5 cm from the bottom of each tube. Use a dropping pipette to add hydrogen peroxide to each tube up to the mark.
2. Punch out 7 disks from a clean coffee filter using a hole punch. Dip one disk into the potato mixture in cup 1. Place the disk in tube 1 and push it to the bottom with a glass rod. When the disk reaches the bottom of the tube, remove the rod and begin timing to determine how long it takes for the disk to float to the surface of the liquid. Record the time in the data table. Repeat the procedure for the samples in cups 2-7. Compute class averages and record them in the data table.
3. Graph the average time versus temperature.

TEACHING TIPS

- Red-skinned and Irish potatoes are the richest in catalase. For comparison, turnips or other tubers could be used.
- The potato mash can be prepared and held for several hours without any significant loss in activity.

- Hydrogen peroxide (3%) can be obtained at any pharmacy or discount store. The plastic medicine cups are available from hospitals or hospital suppliers.
- Sample data tables and graphs are provided following the questions and answers below.

KEY QUESTIONS & ANSWERS

1. According to your graph, at what temperature does catalase begin to lose activity.
Ans. 30°C.
2. At what temperature is catalase completely inactivated?
Ans. 55°C.
3. How could you show that the potato is the source of the catalase enzyme?
Ans. By dipping the filter paper in plain water and testing the activity.
4. Could you imagine other ways, besides heat, of controlling enzymatic activity.
Ans. By altering the pH of the blanch water.
5. Why do we mark a line 5 cm from the bottom of the test tube?
Ans. To have a constant distance for each disk to travel.
6. Would you expect catalase to be inactivated under normal blanching conditions for potatoes (60-65°C)? Why or why not?
Ans. Yes, because catalase is inactivated at 55°C or higher temperatures.

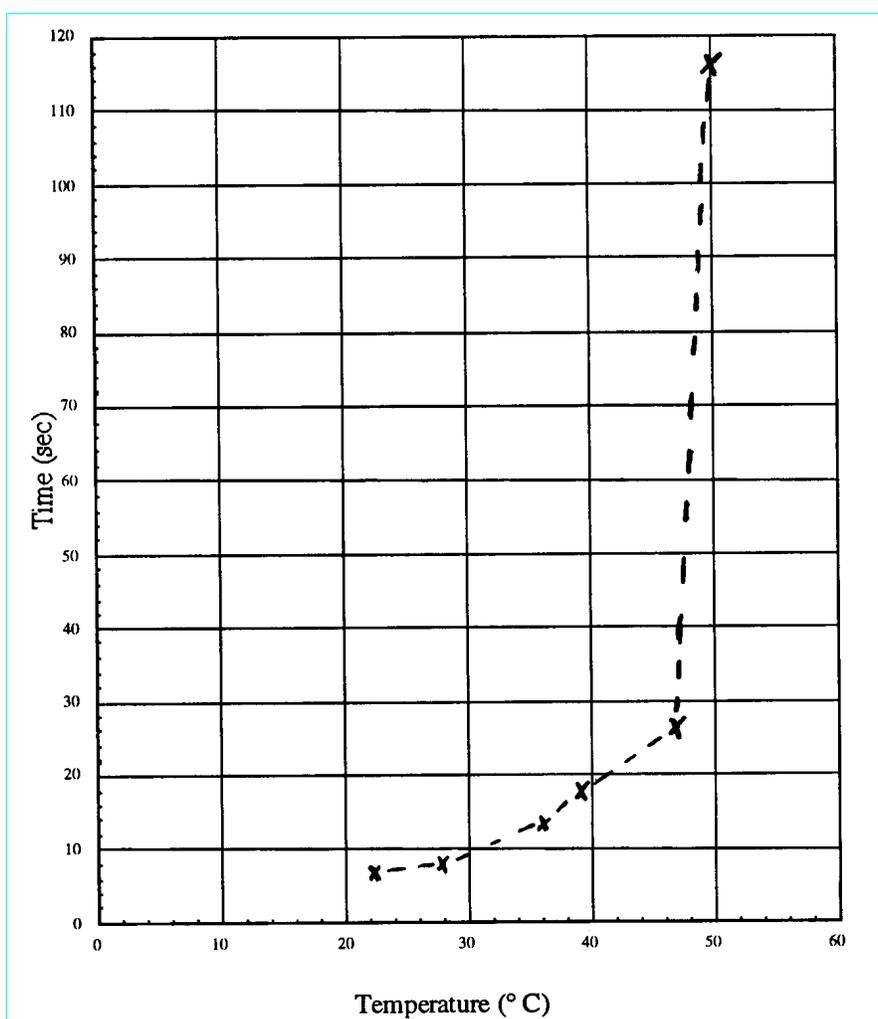
ASSESSMENT STRATEGIES

- Teachers may use a skills checklist, short essay exam or multiple-choice test.
- Students will:
 - Accurately complete data tables.
 - Accurately construct a time-vs-temperature graph.
 - Correctly answer key questions.
 - Conduct the tests carefully and accurately.

Sample Data Tables

The Effect of Heating on the Activity of Catalase in Potatoes

Tube #	Temperature Reached (°C)	Time (sec)	
		Group Data	Class Average
1 (initial)		8	7
2	30	8	7
3	35	14	15
4	40	16	18
5	45	26	25
6	50	118	116
7	55	>120	>120



Visual Master 1

Classes of Enzymes

Proteases (P), Lipases (L), Carbohydrates (C), Other enzymes (O)

Some Desirable Changes Produced by Enzymes

- Formation of cheese curd by rennin (P)
- Meat tenderizing by papain, bromelin, microbial protease (P)
- Contact-lens cleaning by microbial protease (P)
- Protein digestion by pepsin, trypsin (P)
- Development of flavor in cheeses by lipase, esterase (L)
- Increased loaf volume in bread by amylase (C)
- Prevention of staling in bread by amylase (C)
- Fruit ripening by polygalacturonase (C)
- Removal of fruit juice from pulp by pectinase (C)
- Production of soft center in chocolate covered cherries by invertase (C)
- Production of beer by amylase, glucoamylase, protease (C, P)
- Production of light beer by amylase (C)
- Debittering of fruit juices by naringinase (O)
- Production of low- and no-lactose dairy products for persons with lactose intolerance by lactase (C)
- Starch digestion by salivary amylase (C)
- Clarifying of juice drinks by amylase (C)
- Prevention of sloughing in canned green beans by pectin methylesterase (C)

Some Undesirable Changes Produced by Enzymes

- Rotting of foods by protease (P)
- Development of rancidity in fats by microbial lipase (L)
- Over-ripening of fruits by polygalacturonase (C)
- Softening of unblanched vegetables by preoxidase (O)
- Development of off-flavors and off-odors in unblanched vegetables by catalase, lipoxygenase (O)
- Browning of certain fruit and vegetable slices by polyphenolase (O)

Visual Master 2

Figure 1

Two Models of Enzyme Action

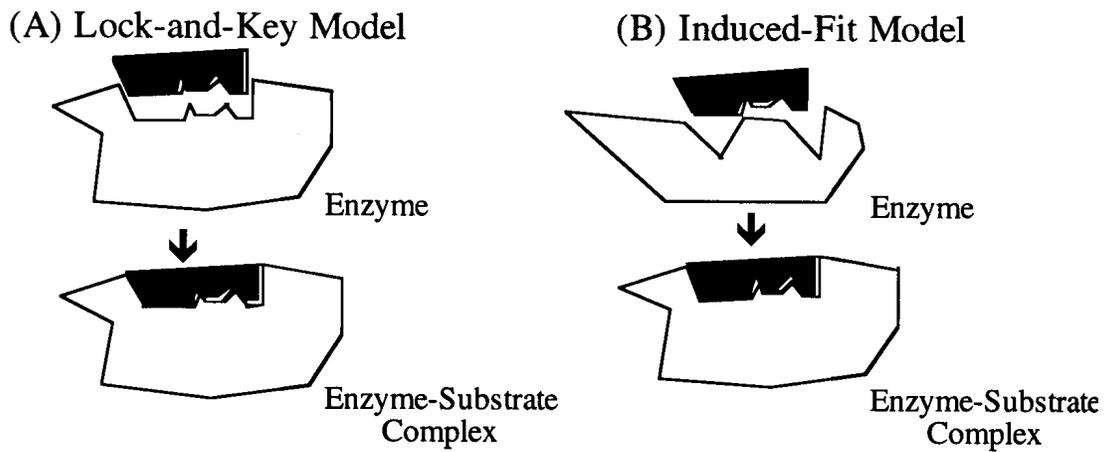
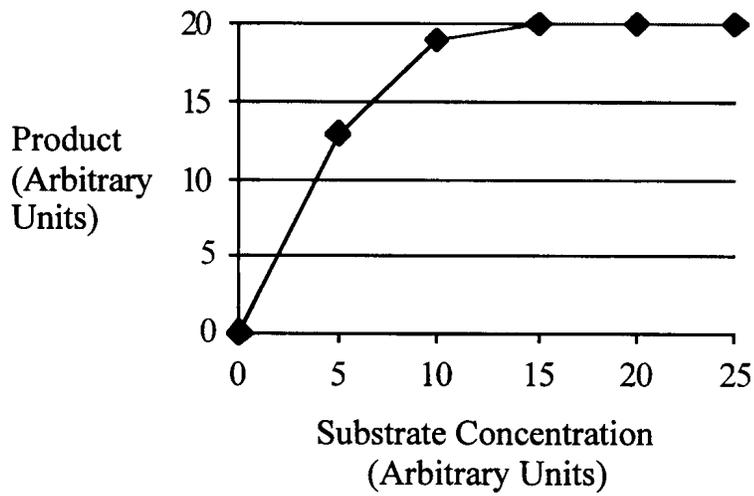


Figure 2

Effect of Substrate Concentration on Activity



Visual Master 3

Figure 3

Effect of Temperature on Activity

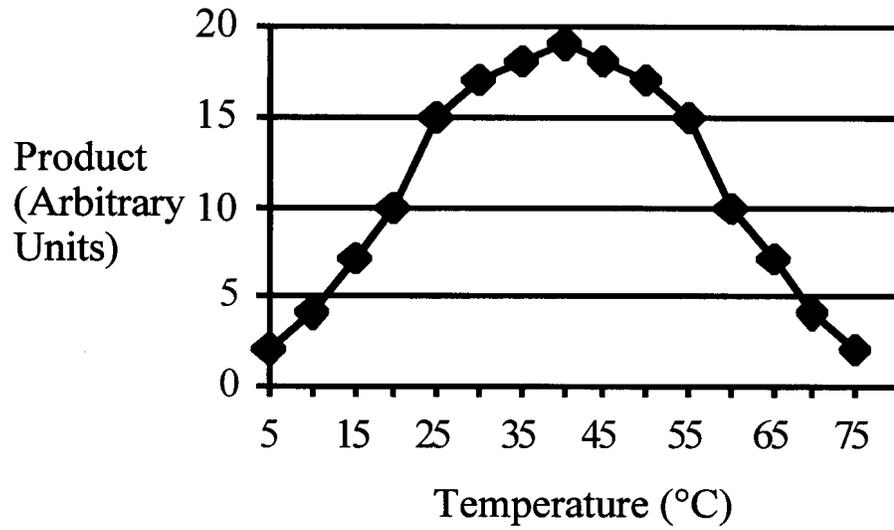


Figure 4

Effect of pH on Activity

