IFT Experiments in Food Science Series

Enzymes in Food Systems

Activity #3

Enzymatic Browning of Apples

A Science Unit for Secondary School Curriculum

Institute of Food Technologists
The Society for Food Science and Technology

TEACHER ACTIVITY GUIDE

Enzymatic Browning of Apples

EXPECTED OUTCOMES

This experiment will illustrate that apple slices exposed to the air will brown readily. Dipping the apples in ascorbic acid, citric acid, or acetic acid or soaking them in water will reduce the level of browning.

ACTIVITY OBJECTIVE

This experiment will illustrate to the student that:

1. Apples contain an enzyme called *polyphenol oxidase* or *phenolase*. In the presence of oxygen from the air, this enzyme catalyzes the formation of brown pigments called *melanins*.
2. Treatment of the apple slices with organic substances such as ascorbic acid, citric acid, or acetic acid will reduce the level of browning. The reduction in browning is dependent on the type of substance and its concentration.
3. Soaking in plain water also reduces the level of browning by restricting the amount of oxygen in contact with the apple tissue.

ACTIVITY LENGTH

Approximately 1 hour

SCIENTIFIC PRINCIPLES

When fruits or vegetables are peeled or cut, enzymes contained in the plant tissue are released. In the presence of oxygen from the air, the enzyme polyphenol oxidase (phenolase) catalyzes one step in the biochemical conversion of plant phenolic compounds to brown pigments known as melanins. This reaction, called enzymatic browning, occurs readily at warm temperatures when the pH is between 5.0 and 7.0.

The presence of iron or copper can increase the rate of the reaction. This can be easily observed when fruit is cut with a rusty knife or mixed in a copper bowl.

Bruising or other injury to the plant tissue disrupts the structural arrangement of constituents within the cells and allows the contents to make contact with oxygen. This may lead to browning of uncooked fruit tissue.

Enzymatic browning can be a significant problem, limiting the shelf life of many fruits and vegetables which have had little heat applied during processing. However, enzymatic browning is not always a defect. The browning reaction contributes to the desirable color and flavor of raisins, prunes, coffee, tea, and cocoa.

Several substances have been used in the food industry to prevent browning of fruits and vegetables:

Sulfites prevent browning by releasing sulfite ions, which prevent melanin formation. These compounds were used extensively until the Food and Drug Administration received reports of adverse allergic-type reactions to foods containing sulfites. FDA requires that the presence of sulfites in foods be declared on the label when the sulfiting agents are used as a preservative regardless of the level used, or when they are used for a technical effect at a level of 10 parts per million (ppm) or more.

Ascorbic acid (vitamin C) acts as an antioxidant. Oxygen preferentially reacts with the ascorbic acid, rather than with the phenolic compounds in the fruit or vegetable. Browning does not proceed until all the ascorbic acid is used up in the reaction.

Citric acid and *acetic acid* lower the pH of the fruit tissue to retard the action of the polyphenol oxidase. If the pH is reduced below 3.0, the polyphenol oxidase will be inactivated. Citric acid also acts as a chelating agent, tying up copper ions which are necessary cofactors in the reaction.

Placing fresh fruit in a water bath, blanching, will temporarily inhibit the browning reaction, since water restricts the amount of oxygen in contact with fruit tissues. Heating also prevents browning by inactivating the polyphenol oxidase. Since heat also cooks the fruit, heating cannot be used as a treatment for fruits which will be served fresh.

VOCABULARY

- *Antioxidant*—a substance that by-passes oxidation or inhibits reactions promoted by oxygen; often used as a preservative.
- *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.
- *Chelating agent*—a substance that combines with a metal ion (e.g., copper) and prevents it from reacting with other substances.
- *Enzymatic browning*—a biochemical process in which fruit or vegetable tissues turn brown when exposed to oxygen. This process is catalyzed by polyphenol oxidase.
- *Melanin*—any of a group of brown or black pigments occurring in plants and animals.
- *Polyphenol oxidase*—a copper-containing enzyme, also called phenolase, that catalyzes the oxidation of phenolic compounds contained in plant tissues.

MATERIALS REQUIRED

- Fresh apple slices of approximately the same size (6 per group)
- Test solutions for dipping:
  0.1% Ascorbic acid
  0.1% Citric acid
  0.1% Acetic acid
  1.0% Acetic acid
- Beaker or cup with water
- Tongs
- Paper towels

INSTRUCTIONAL STRATEGIES AND PROCEDURES

1. Place an untreated apple slice on a paper towel. Label the towel "Control."
2. Using tongs, dip another apple slice into one of the test solutions for 30 seconds, place it on the towel, and label the towel with the name of the solution. Rinse the tongs and repeat the same procedure for the other three solutions.
3. Soak one slice in water for 30 seconds. Place it on a towel and label the towel "Water Soak."
4. Note the time and temperature in your data table. Observe the slices every 10 minutes for one hour and record your observations. Compare your results with those obtained by the rest of the class.

TEACHING TIPS

- Bananas, peaches, pears, and avocados also work well. If a rusty knife is used to cut the fruit, the rate of browning will be increased.
- Preparation of test solutions:
  •*0.1% Ascorbic acid*: Dissolve a 200-mg vitamin C tablet in 200 mL of water.
  •*0.1% Citric acid*: Mix 0.2 g of citric acid with 200 mL of water. Pure citric acid can usually be purchased in the home canning section of your grocery store. If not, it may be purchased from Home Canning Supply & Specialties, 2117 Main St., Ramona, CA 92065 (phone 619-788-0520). [If pure citric acid is not available, lemon juice may be substituted. Mix 5 mL of lemon juice (containing 4% citric acid) with 195 mL of water.]
  •*0.1% Acetic acid*: Mix 4 mL of vinegar (containing 5% acetic acid) with 196 mL of water.
  •*1.0% Acetic acid*: Mix 40 mL of vinegar (containing 5% acetic acid) with 160 mL of water.
- Place each solution in a labeled beaker or wide-mouth jar.
- Students should prepare a bar graph showing the level of browning after 60 minutes.
- A sample data table follows the questions and answers below.

KEY QUESTIONS & ANSWERS

1. What causes browning when fresh fruits and some vegetables are peeled or cut?

ans. The browning is caused by conversion of phenolic compounds to melanins catalyzed by the enzyme polyphenol oxidase.

2. What conditions enhance the browning process? Why?

ans. Oxygen—required for the reaction to occur.
Heat—browning increases with increasing temperature until the polyphenol oxidase is inactivated.
pH—the optimal pH for the browning reaction is between 5.0 and 7.0. If the pH is below 3.0, the enzyme will be inactivated.
Metal ions—copper is necessary for the action of the polyphenol oxidase; other metal ions, such as iron, also increase the rate of the reaction.

3. How do food additives or treatment processes in use today prevent or retard browning in fruits and vegetables?

ans. Sulfites—inhibit melanin formation.
Ascorbic acid (vitamin C)—prevents oxygen from reacting with the polyphenol oxidase.
Citric acid and acetic acid—lower the pH, which decreases the enzyme activity. Citric acid also ties up copper ions and prevents them from participating in the reaction.
Heating—inactivates the polyphenol oxidase.

4. Why do citrus juices retard browning in fresh fruits?

ans. Because ascorbic acid and citric acid are present in the fruit juices.

ASSESSMENT STRATEGIES

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

Sample Data Tables

Enzymatic Browning of Apple Slices

Level of Browning*						
Time (min)	No Treatment (Control)	Ascorbic Acid (0.1%)	Citric Acid (0.1%)	Acetic Acid (0.1%)	Acetic Acid (1.0%)	Water Soak
0	1	1	1	1	1	1
10	1	1	1	1	1	1
20	2	1	1	1	1	2
30	2	2-3	1	1	1	2
40	3-4	2-3	2-3	2-3	1	3-4
50	3-4	2-3	2-3	2-3	1	3-4
60	3-4	3-4	2-3	2-3	1	3-4

* **5=Completely dark brown**
4=Fully covered light brown
3=Half-covered light brown
2=Slight or scant brown patches
1=No browning present

Time started: 1:10 p.m. **Temperature:** 26°C

IFT User Survey

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Once completed you may mail it to:

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Thank you for your help!

*** *** *** *** *** ***

1. What grade(s) and subjects do you teach?

   Grade(s):_____ Subject(s):_____

2. How many students participated in this lesson? _____

3. Please enter the name of the experiment or workbook you are evaluating.

   _____

4. On a scale of 1 – 10 (10 = most acceptable), how do you evaluate the relationship of the materials to understanding of science concepts? (Circle one.)

   1 2 3 4 5 6 7 8 9 10

5. On a scale of 1 – 10 (10 = most acceptable), how do you evaluate the understanding of the materials by your students? (Circle one.)

   1 2 3 4 5 6 7 8 9 10

6. Please describe any unusual or interesting way in which you presented this material to your students.

   _____
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   _____
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7. We would appreciate any additional comments or suggestions you may have…

   _____
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Thank You!

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