

Enzymatic Browning of Apples



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ACTIVITY

The purpose of this experiment is to monitor the level of enzymatic browning of apple slices. Apples contain an enzyme called *polyphenol oxidase (phenolase)*

In the presence of oxygen from the air, this enzyme catalyses the formation of brown pigments called melanins. Treatment of the apple slices with 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. Soaking in water alone will temporarily reduce the level of browning by restricting the amount of oxygen in contact with the apple slices.

BACKGROUND INFORMATION

When fruits or vegetables are peeled or cut, enzymes contained in the plant cells are released. In the presence of oxygen from the air, the enzyme *phenolase* catalyses one step in the biochemical conversion of plant phenolic compounds to form 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 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 arrangement of chemicals within the cells and allows these chemicals to make contact with oxygen. This may lead to browning of uncooked fruits. 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 colour and flavour of raisins, prunes, coffee, tea, and cocoa.



NSW Syllabus References:

Biology HSC syllabus
Core Topic 9.2 Maintaining a Balance

Students:

- Identify data sources, plan, choose equipment or resources and perform a first-hand investigation to test the effect of
 - increased temperature
 - change in pH
 - change in substrate concentration on the activity of named enzyme(s)

NSW Syllabus References:

Years 7-10 Science syllabus

- Student Research project
- Prescribed Focus Area – application and uses of science

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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. The Australia New Zealand Food Standards Code permits the presence of sodium or potassium sulfites in apples for manufacturing purposes to a level of 200mg/kg (ppm). Sulfites are not allowed in foods in Australia unless expressly permitted by the Foods Standards Code.

Ascorbic acid (vitamin C) acts as an antioxidant. Oxygen is more likely to react with the ascorbic acid, rather than with the phenolic compounds in the fruit or vegetable. Browning does not proceed until most of 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 *phenolase*. If the pH is reduced below 3.0, the activity of the *phenolase* will be severely inhibited.

Placing fresh fruit in a water bath 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 *phenolase*. Since heat also cooks the fruit, heating cannot be used as a treatment for fruits which will be served fresh.

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.
3. Rinse the tongs and repeat the same procedure for the other three solutions.
4. Soak one slice in water for 30 seconds. Place it on a towel and label the towel "Water Soak."
5. Note the time and temperature in your data table. Observe the slices every 10 minutes for one hour and record your observations.
6. Compare your results with those obtained by the rest of the class.



MATERIALS

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

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Results

Data table
Enzymatic Browning of Apple Slices

Time (min.)	Level of browning					
	No treatment (control)	Ascorbic acid (0.1%)	Citric Acid (0.1%)	Acetic Acid (0.1%)	Acetic Acid (1.0%)	Water soak
0						
5						
10						
15						
20						
25						
30						
35						
40						
45						
50						

Time started:

Temperature at start:

Key

- 5 = completely dark brown
- 4 = fully covered light brown
- 3 = half-covered light brown
- 2 = some brown patches
- 1 = no browning present

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KEY QUESTIONS & ANSWERS

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

A. *The browning is caused by the conversion of phenolic compounds to melanins catalysed by the enzyme polyphenol oxidase.*

Q. What conditions promote the browning process? Why?

A. *Oxygen is 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 significantly below 3.0 the enzyme will be inactivated.
Metal ions – such as iron and copper can increase the rate of the reaction.

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

A. *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.

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

A. *Because ascorbic acid and citric acid are present in the fruit juices and they lower the pH which may reduce the activity of the enzyme.*