Investigating what makes fruit go brown

The purpose of this activity is:

* to observe the situations in which a sample of apple or potato becomes brown
* to test a number of hypotheses that might explain what causes the reaction
* to apply what is learned to the commercial processes of food production

### **Procedure**

SAFETY:

Avoid skin contact with benzene-1,2-diol or phenol.

Wear eye protection when handling acids and the other chemicals.

Beware of sulfur dioxide emissions from sodium hydrogensulfate(IV) solution – keep your nose well away!

***Initial observation:***

When apple or potato is broken, it tends to break between cells rather than through cells. Crushing produces extensive tissue damage and mixes together enzymes and cell contents that would normally be kept apart. It also allows rapid diffusion of oxygen through the sample. Intact plant cells use up oxygen as the cells release energy. Once damaged, cells no longer use up oxygen in this way.

1. Cut an apple or potato into eight pieces. Take one piece and crush it in the mortar and pestle. Put the crushed sample on a tile next to an uncrushed piece. Compare the rate at which both samples go brown.
2. When the uncrushed piece is brown, **cut** a small piece from it and also **break** a small piece from it. Note the colour of the freshly-exposed surfaces.
3. Note which browns more quickly – the cut or broken surface.

### **Questions about *Initial observation***

1. Put these samples in order of browning, most rapid first.
   * cut surface
   * broken surface
   * crushed sample
2. What ideas do you have about what might be causing this browning reaction? How could you test those ideas?

***Investigation 1: Is the reaction an effect of microorganisms acting on the tissue?***

A solution of 1% phenol will kill any microorganisms present, but will not activate enzymes in the plant tissue.

1. Take two slices of apple or potato.
2. Soak one in a 1% solution of phenol for 1 minute.
3. Soak a similar slice in pure water as a control.
4. Use tongs to remove the slices, shake off excess liquids. Wash one surface of each with 3-4 drops of benzene-1,2-diol solution. Observe the rate of browning of each slice.

### **Questions about *Investigation 1:***

1. Which slice browns most rapidly?
2. Is there evidence that this reaction is caused by microorganisms?
3. Would you expect to find more microorganisms on the surface of fruit and vegetables or within the tissue?

***Investigation 2: Do enzymes control the browning reaction?***

This investigation involves observing the rate of reaction at different temperatures. Think about how you expect enzyme reactions to be affected by changing temperature.

1. Take four slices of apple or potato. Treat as described below:

* Immerse in a water bath at 100 °C for 1 minute.
* Immerse in a water bath at 60 °C for 1 minute.
* Immerse in a water bath at 40 °C for 1 minute.
* Immerse in water at room temperature for 1 minute.

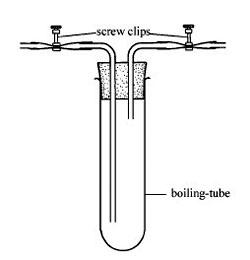
1. Remove the slices using tongs, shake off excess liquid. Wash the surfaces with 3-4 drops of benzene-1,2-diol.
2. Compare the rates at which the four samples go brown.

### **Questions about *Investigation 2:***

1. Is there any evidence that this reaction is controlled by enzymes?
2. What is the effect of heating above 60 °C on an enzyme?
3. Is there any disadvantage in using heat treatment to preserve the colour of foodstuffs?

### ***Investigation 3: Does the reaction require air or some part of the air?***

Use the apparatus below to subject each sample to a different kind of atmosphere.



1. Put four slices of apple or potato in four separate boiling tubes.
2. Wash the upper surfaces with 3-4 drops of 1% benzene-1,2-diol.
3. Use the apparatus provided to hold the apple or potato slices in different atmospheres as described below:
   * an atmosphere of nitrogen

* an atmosphere of carbon dioxide
* normal atmospheric air

1. Note the rate at which each slice goes brown.

### **Questions about *Investigation 3:***

1. Which component in the air seems to be involved in browning?
2. Food, such as potato crisps are often “packed in a protective atmosphere” (read packets). What might be the main component of such an atmosphere?

### ***Investigation 4: How does ascorbic acid affect the browning reaction?***

Ascorbic acid is a reducing agent. So it can reverse an oxidation reaction – it is an ‘antioxidant’. Ascorbic acid is commonly known as vitamin C.

1. Take six slices of apple or potato.
2. Soak each slice in one of the following solutions for 2 minutes:

* 5% ascorbic acid
* 3.5% ascorbic acid
* 2.5% ascorbic acid
* 2% ascorbic acid
* 1% ascorbic acid
* distilled water

1. Remove the slices, shake off excess solution and wash the upper surfaces with 3-4 drops of 1% benzene-1,2-diol.
2. Arrange the tubes in sequence to show which goes brown first and which last.

### **Questions about *Investigation 4:***

1. Does ascorbic acid stop or slow the browning process?
2. How does changing concentration of ascorbic acid affect the browning reaction?
3. Do you think ascorbic acid could restore the fresh colour to a slice that is already brown? (Test one to see.)
4. What do you think about using ascorbic acid as an additive in food to prevent oxidation?

### ***Investigation 5: How do other chemicals affect the browning reaction?***

Some chemicals will change the conditions to make them no longer optimal for enzyme action, or by denaturing the enzyme. Some will prevent oxidation by removing oxygen. Some will reverse the oxidation reaction by reducing the products back to the original chemicals. Think about how each of these treatments affects apples or potatoes as a foodstuff.

1. Take seven slices of apple or potato.
2. Soak each in one of the following solutions for 2 minutes:

* 2% hydrochloric acid
* 2% citric acid
* 2% sodium hydrogensulfate(IV)
* 2% sodium chloride
* 2% sucrose
* boiling water
* cold distilled water

1. Remove the slices, shake off excess solution and wash the upper surfaces with 3-4 drops of 1% benzene-1,2-diol.
2. Note how soon each slice goes brown.

### **Questions about *Investigation 5:***

1. Which methods are effective at controlling browning?
2. How do you think each method works?
3. Which methods would be acceptable for treating foodstuffs? Why, or why not?
4. Is sodium chloride (salt) or sucrose (sugar) better for treating apple in a commercial situation? Why? Which would be better for treating potatoes?

### **Answers to *Initial observation* questions:**

When apple or potato is broken, it tends to break between cells rather than through cells. Crushing produces extensive tissue damage and mixes together enzymes and cell contents that would normally be kept apart. It also allows rapid diffusion of oxygen through the sample. Intact plant cells use up oxygen as the cells release energy. Once damaged, cells no longer use up oxygen in this way.

1. In order of browning, the samples should be first the crushed sample, then the cut, then the broken sample.
2. Students could have many ideas (or none!) about what might be causing this browning reaction. Testing the ideas by inactivating enzymes, or by removing microorganisms or air would be good starting points even if they are unsure of exactly how to go about it.

**Answers to *Investigation 1* questions*:***

A solution of 1% phenol will kill any microorganisms present, but will not activate enzymes in the plant tissue.

1. Both slices should brown at a similar rate.
2. There should be no evidence that this reaction is caused by microorganisms – application of phenol to kill any microorganisms does not affect browning rate.
3. You would expect to find more microorganisms on the surface rather than within the tissue.

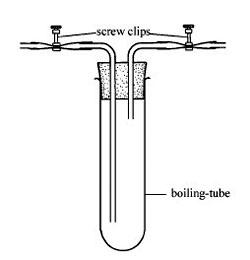
**Answers to *Investigation 2* questions*:***

This investigation involves observing the rate of reaction at different temperatures. Enzyme reactions are sensitive to temperature, operating best at an optimum (usually 30- 40 °C) and not at all at very high temperatures.

1. The evidence that this reaction is controlled by enzymes is that the slice at 40 °C browns most quickly and those heated above 60 °C do not brown. If it were a simple chemical reaction, you would expect it to be faster at higher temperatures.
2. Heating above 60 °C will denature an enzyme and make it inactive.
3. Heat treatment will also change the physical properties of the food (in the same way as cooking it does) which might not be what the preserving process is trying to achieve.

### **Answers to *Investigation 3* questions:**

Use the apparatus below to subject each sample to a different kind of atmosphere.



1. Oxygen in the air seems to be involved in the browning reaction.
2. Food, such as potato crisps are often “packed in a protective atmosphere” (read packets). Nitrogen might be the main component of such an atmosphere.

### **Answers to *Investigation 4* questions:**

Ascorbic acid is a reducing agent. So it can reverse an oxidation reaction – it is an ‘antioxidant’. Ascorbic acid is commonly known as vitamin C.

1. Ascorbic acid slows the browning process.
2. The higher concentrations of ascorbic acid delay browning for longer.
3. Yes. And it can. (Test one to see.)
4. Ascorbic acid is a good additive in food to prevent oxidation because it is a necessary micronutrient/ vitamin in the human diet.

### **Answers to *Investigation 5* questions:**

Some chemicals will change the conditions to make them no longer optimal for enzyme action, or by denaturing the enzyme. Some will prevent oxidation by removing oxygen. Some will reverse the oxidation reaction by reducing the products back to the original chemicals.

1. All the methods (except cold distilled water, the control) are effective at controlling browning.
2. The methods work like this:

| **Method of controlling browning** | **How does it work?** |
| --- | --- |
| hydrochloric acid | Makes pH not optimum for enzyme |
| citric acid | Makes pH not optimum for enzyme |
| sodium hydrogensulfate(IV) | Produces antioxidant (sulfur dioxide) |
| sodium chloride | Makes ionic concentration not optimum for enzyme |
| sucrose | Reduces solubility of oxygen at cell surface |
| boiling water | Denatures enzyme |

1. Citric acid, sodium chloride and sucrose are definitely suitable for treating foodstuffs as all, at certain levels, are present in our food anyway. Hydrochloric acid is too strong an acid to be safe in foods. Sulfur dioxide is safe at low concentrations, but some people are sensitive to it. Boiling water is OK, but a 1 minute treatment is not long enough on its own to preserve foods for a long time.
2. Sucrose (sugar) better for treating apple in a commercial situation, because the flavours work well together. Salty apple would maintain its colour but taste really bad. Either might work for potato, but most people prefer salty potato rather than sugary.