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**Department Of Biology and Biochemistry**

**BIOL111**

**Expt. #5:What Makes Fruits Go Brown ?**

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**What Makes Fruits Go Brown ?**

**Objectives:**

To determine the reasons behind going fruits brown by chemical method and to observe the situations in which a sample of apple becomes brown.

**Introduction:**

**Browning** is the process of [fruit](https://en.wikipedia.org/wiki/Food) turning brown due to the chemical reactions that take place within. The process of [fruit](https://en.wikipedia.org/wiki/Food) browning is one of the most important reactions that take place in [fruit](https://en.wikipedia.org/wiki/Food) chemistry and represents an interesting research topic regarding health, nutrition, and food technology. Though there are many different ways food chemically changes over time, browning in particular falls into main 2 categories; enzymatic and non-enzymatic processes. The browning process of foods may yield desirable or undesirable results, depending on the type of food. Browning has many important implications on the food industry relating to nutrition, technology, and economic cost. Of particular interest to many researchers studying the food browning processes is the control (inhibition) of browning and the different methods that can be employed to maximize this inhibition and ultimately prolong the shelf life of food.

**Enzymatic browning** is one of the most important reactions that takes place in most fruits and vegetables as well as in seafood. These processes affect the taste, color, and value of such foods. Generally, it is a chemical reaction involving [polyphenol oxidase](https://en.wikipedia.org/wiki/Polyphenol_oxidase), [catechol oxidase](https://en.wikipedia.org/wiki/Catechol_oxidase), and other [enzymes](https://en.wikipedia.org/wiki/Enzyme) that create [melanins](https://en.wikipedia.org/wiki/Melanin) and [benzoquinone](https://en.wikipedia.org/wiki/Benzoquinone) from [natural phenols](https://en.wikipedia.org/wiki/Natural_phenol). Enzymatic browning (also called oxidation of foods) requires exposure to [oxygen](https://en.wikipedia.org/wiki/Oxygen). It begins with the oxidation of [Phenols](https://en.wikipedia.org/wiki/Phenols) by [Polyphenol oxidase](https://en.wikipedia.org/wiki/Polyphenol_oxidase) into [Quinones](https://en.wikipedia.org/wiki/Quinone), whose strong electrophilic state causes high susceptibility to a nucleophilic attack from other proteins. These Quinones are then polymerized with other phenols and enzymes ([Catechol oxidase](https://en.wikipedia.org/wiki/Catechol_oxidase)) in a series of reactions, eventually resulting in the formation of brown pigments on the surface of the food. The rate of enzymatic browning is reflected by the amount of active polyphenol oxidases present in the food. Hence most research investigating methods to inhibit enzymatic browning has focused on hindering polyphenol oxidase activity. However, not all browning of food produces negative effects. A few examples are listed below:

Enzymatic browning may be beneficial for:

* Developing color and flavour in [Coffee](https://en.wikipedia.org/wiki/Coffee), [Cocoa beans](https://en.wikipedia.org/wiki/Cocoa_bean), and [tea](https://en.wikipedia.org/wiki/Tea).
* Developing color and flavour in [dried fruit](https://en.wikipedia.org/wiki/Dried_fruit) such as [figs](https://en.wikipedia.org/wiki/Ficus) and [raisins](https://en.wikipedia.org/wiki/Raisin).

Enzymatic browning negatively affects:

* Fresh fruit and vegetables, including [apples](https://en.wikipedia.org/wiki/Apple), [potatoes](https://en.wikipedia.org/wiki/Potato), [bananas](https://en.wikipedia.org/wiki/Banana) and [avocados](https://en.wikipedia.org/wiki/Avocado).
* Polyphenols oxidases is the major biochemical reaction in the formation of [Melanosis](https://en.wikipedia.org/wiki/Melanosis) in crustaceans such as shrimp.

**Method:**

**Initial Observation**: The apple was cut into eight equal pieces. One piece was taken and crushed in the mortar and pestle. The crushed sample was put on a tile next to an uncrushed piece. **The rate at which both samples go brown was compared**. The uncrushed piece was cut when it becomes brown using a knife. Small piece of it was broken using a hand. **The**

**color of the freshly exposed surfaces was noted**.

**Investigation 1** : Two apple slices were taken, one of them was soaked in 1% phenol solution to kill microorganisms for 1 minute. The other piece was soaked in pure water as a control. The slices were removed using tongs and shaken off excess liquids. The surface was washed with 4 drops of catechol. **The rate of browning of each slice was observed.**

**Investigation 2** : Four apple slices were taken and treated as described below:

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

The slices were removed by tongs and shaken off excess liquids. The surface was washed with 4 drops of catechol. **The rate at which the four samples go brown was compared.**

**Investigation 4**: Six apple slices were taken and each slice was soaked in one of the following solution for 2 minute:

* 5% ascorbic acid.
* 2.5% ascorbic acid.
* 1.5% ascorbic acid.
* 1% ascorbic acid.
* 0.5% ascorbic acid.
* Distilled water.

The slices were removed, shaken off excess solution and the upper surfaces were washed with 4 drops of catechol. The tubes were arranged in sequence to show which goes brown first and which last.

**Investigation 5**: Seven apple slices were taken and each one of them was soaked in one of the following solutions for 2 minute:

* 2% Hydrochloric acid.
* 2% Citric acid.
* 2% Sodium hydrogensulfate (IV).
* 2% Sodium chloride.
* 2% Sucrose.
* Boiling water.
* Cold distilled water.

The slices were removed, shaken off excess solution and the upper surfaces were washed with 4 drops of catechol. How each slice goes brown was noted.

**Discussion:**

### The crushed sample of apple is faster than uncrushed. The cut surface is faster than broken surface. When apple 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 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. The error was in the left cut apples exposed to the air that contain oxygen.(Initial Observation)

Phenol (C6H6O) and Water both brown at a similar rate. The results indicate to that the microorganisms do not affect the browning. We would expect to find more microorganisms on the surface rather than within the tissue. There should be no evidence that this reaction is caused by microorganisms, application of phenol to kill any microorganisms does not affect browning rate.(Investigation 1)

The enzymes is controlled by temperature. The high temperature will denature an enzyme and make it inactive. The enzymes control the reaction as we see.( Investigation 2)

Ascorbic acid is commonly known as vitamin C, ascorbic acid is a reducing agent, so it can reverse an oxidation reaction. The browning reaction is affected by ascorbic acid it works to slows the browning process. When we changing concentration of ascorbic acid this will affect on the speed of the browning process, when we increase the concentration of ascorbic acid this will delay browning for longer and when we decrease the concentration of ascorbic acid this will increase the speed of browning.( Investigation 4)

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. Chemicals is used by us **(Hydrochloric acid, citric acid, sodium chloride, sucrose, sodium hydrogensulfate (IV) ,boiling water, cold distilled water)**,all thesechemicals (expect cold distilled water-the control-)are effective at controlling browning.(Investigation 5)

**Conclusion:**

1-The browning reaction is controlled by enzymes.

2-The browning reaction is affected by concentration of ascorbic acid.

3-The browning reaction is affected by chemical substance.

4-The browning reaction is not impacted by microorganisms.

**Data and Results:**

I collected samples of apples in different investigations to know what controls the browning of apple. We used Catechol (C6H6O2 ) to speed up the interaction or process. I observed that there are more microorganisms on the surface rather than within the tissue. The browning reaction is determined by temperature (°C). 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. 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.

**Initial Observation:**

**Question**: Put these samples in order of browning, most rapid first.

* + cut surface
  + broken surface
  + crushed sample

**Answer:** In order of browning, the samples should be first the crushed sample, then the cut, then the broken sample.

**Investigation 1 :**

| **Materials** | **Result** |
| --- | --- |
| Phenol(C6H6O ) | Both of them should brown at a similar rate |
| Water |

**Figure 1.1**

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

**Investigation 2:**

Temperature °C:

100°C < 60°C < 40°C < Room Temperature

| **Temperature °C** | **Result** |
| --- | --- |
| 100°C | - |
| 60°C | - |
| 40°C | + |
| Room Temperature | + |

**Figure 1.2**

Heating above 60°C will denature an enzyme and make it inactive.

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.

**Investigation 4:**

5% Ascorbic acid < 2.5% Ascorbic acid< 1.5% Ascorbic acid< 1% Ascorbic acid< 0.5% Ascorbic acid< Distilled water

Ascorbic acid slows the browning process. The higher concentrations of ascorbic acid delay browning for longer

**Investigation 5:**

| **Solutions** | **Result** | **Work** |
| --- | --- | --- |
| 2% Hydrochloric acid | - | Makes pH not optimum for enzyme |
| 2% Citric acid | - | Makes pH not optimum for enzyme |
| 2% Sodium hydrogensulfate (IV) | - | Produces antioxidant (sulfur dioxide) |
| 2% Sodium chloride | + | Makes ionic concentration not optimum for enzyme |
| 2% Sucrose | + | Reduces solubility of oxygen at cell surface |
| Boiling water | + | Denatures enzyme |
| Cold distilled water | - | Controls |

**Figure 1.3**

(+):The browning reaction is occur.

(-):The browning reaction is not occur.

**References:**

**1- Introduction-** [**https://en.wikipedia.org/wiki/Food\_browning**](https://en.wikipedia.org/wiki/Food_browning)

[**http://io9.gizmodo.com/why-does-your-apple-turn-brown-and-how-can-you-stop-it-1605149639**](http://io9.gizmodo.com/why-does-your-apple-turn-brown-and-how-can-you-stop-it-1605149639)

**2- Discussion-** [**http://www.nuffieldfoundation.org/practical-biology/investigating-what-makes-fruit-go-brown**](http://www.nuffieldfoundation.org/practical-biology/investigating-what-makes-fruit-go-brown)

**Appendix:**

**1-** The chemical reaction between oxygen from the air with an enzyme located it in the fruit to turn the fruit brown. can be simplified to:

**Polyphenol Oxidase + O2 → Melanin (Brown Color)**

**2-** **Definition of an enzyme: A substance produced by all living organisms that speed up a chemical reaction (i.e. speeding up the browning of fruit).**

**• FACT: Enzymes usually end with the suffix -ase.**

(Q): Which component in the air seems to be involved in browning?

(A): Oxygen in the air seems to be involved in the browning reaction.

(Q): Food, such as potato crisps are often “packed in a protective atmosphere” (read packets). What might be the main component of such an atmosphere?

(A): Nitrogen might be the main component of such an atmosphere.