# Lab (7): Effect of various factors on polyphenol oxidase activity

## Introduction:

A substance that speeds up a chemical reaction—without being consumed—is called a catalyst. The catalysts for biochemical reactions that occur in the living organisms are called **enzymes**.(1) Enzymes are the most remarkable and highly specialized proteins. They extraordinary catalytic power, often far greater than that of synthetic or inorganic catalysts. Enzymes have a high degree of specificity for their substrates, they accelerate chemical reactions tremendously, and they function in aqueous solutions under very mild conditions of temperature and pH. (2)

Enzymes perform the critical task of lowering a reaction's activation energy—that is, the amount of energy that must be supplied for a reaction to begin. Enzymes work by binding to reactant molecules and holding them in such a way that the chemical bond-breaking and bond-forming processes take place more readily. (1) The distinguishing feature of an enzyme-catalyzed reaction is that it takes place within the confines of a pocket on the enzyme called the **active site**.(2) To catalyse a reaction, an enzyme will bind to one or more reactant molecules. These molecules are the enzyme's **substrates**. In some reactions, one substrate is broken down into multiple products. In others, two substrates come together to create one larger molecule.(1) A simple enzymatic reaction might be written (2) :

$$\mathbf{E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P}$$

Where E, S, and P represent the enzyme, substrate, and product; ES and EP are transient complexes of the enzyme with the substrate and with the product.(2)

For many years, scientists thought that enzyme-substrate binding took place in a simple "**lock-and-key**" fashion. This model asserted that the enzyme and substrate fit together perfectly in one instantaneous step. However, current research supports a more refined view called **induced fit**. As the enzyme and substrate come together, their interaction causes a mild shift in the enzyme's structure that confirms an ideal binding arrangement between the enzyme and the substrate. This dynamic binding maximizes the enzyme's ability to catalyze its reaction.(3)

Many enzymes have been named by adding the suffix "-ase" to the name of their substrate or to a word or phrase describing their activity. The study of enzymes has immense practical importance. In some diseases, especially inheritable genetic disorders, there may be a deficiency or even a total absence of one or more enzymes. For other disease conditions, excessive activity of an enzyme may be the cause. Measurements of the activities of enzymes in blood plasma, erythrocytes, or tissue samples are important in diagnosing certain illnesses.

In addition, enzymes are important practical tools, not only in medicine but in the chemical industry, food processing, and agriculture. [2]

Enzyme browning is a usual phenomenon that can be observed commonly in fruits and vegetables, which results in quality loss of the food including the change in color, taste, flavor, and nutritional value. This occurs when the phenolic compounds present in them react with polyphenol oxidase. Polyphenol oxidase (PPO) is a copper-containing enzyme that catalyse the oxidation of dihydroxy-and trihydroxy phenol to corresponding quinone which has a brown color. [4] In this lab, activity of polyphenol oxidase extracted from potato will be examined qualitatively

## Experiment (1). Examine the protein nature of polyphenol oxidase:

### Aim:

- Examine the protein nature of polyphenol oxidase by biuret test.

### Principle:

Majority of enzymes are proteins. [5] Detection of protein nature will be done using biuret reagent, where the peptide bonds in the proteins (enzymes) treated with an alkaline solution of dilute copper sulphate CuSO4 (biuret reagent) forming a purple coloured complex.[6]

### Materials:

**Chemical**

Potato crude extract, biuret reagent, distilled water.

**Equipment and Glassware**

Test tubes, rack, pipette, pipette pump, water bath.

### Protocol:

1. Label a test tube and add 1ml of enzyme crude extract.
2. Add 2 ml of biuret reagent.

### Results:

| Tube | Observation |
|---|---|
| Enzyme crude extract + biuret reagent | |

## Experiment (2). Test the activity of polyphenol oxidase:

### Aim:

- To demonstrate activity of the enzyme.
- To investigate the effect of incubation time on enzyme activity.

### Principle:

Polyphenol oxidase activity will be examined qualitatively be following the change in the color. The oxidation-reduction reaction that catalysed by this enzyme is accompanied by a color change i.e browning (the produced quinones spontaneously polymerize to form dark-colored phytomelanins) (Figure 1). The intensity of the brown color is proportional to the enzyme's activity. [7]

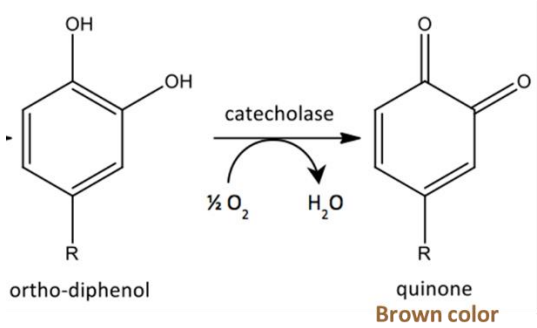


**Figure 1. Oxidation-reduction reaction catalysed by polyphenol oxidase.** [7]

### Materials:

**Chemical**

Potato crude extract, 0.01M catechol, distilled water.

**Equipment and Glassware**

Test tubes, rack, pipette, pipette pump, water bath.

### Protocol:

1. Label 3 test tube as **A**, **B** and **C**.
2. **In tube A (control)**: add 15 drops of the enzyme and 15 drops of catechol.
3. **In tube B**: add 15 drops of the enzyme and 15 drops of distilled water.
4. **In tube C**: add 15 drops of distilled water and 15 drops of catechol.
5. Shake the tubes well.
6. Place all the tubes in the water bath at 37 ºC. Shake each tube every 5 minutes to aerate, thereby adding oxygen to the solution.

### Results:

| Incubation time (Minutes) | Degree of color intensity (Symbol: −, +, ++ or +++) | | |
|---|---|---|---|
| | A | B | C |
| 0 | | | |
| 5 | | | |
| 10 | | | |
| 15 | | | |
| 20 | | | |
| 25 | | | |

## Experiment (3). Demonstrate the chemical nature of polyphenol oxidase:

### Aim:

- To examine the chemical nature of polyphenol oxidase.

### Principle:

Polyphenol oxidase is a protein in nature, and thus effected by various factors that affect proteins. Applying an external denaturation factors or compound such as a strong acid or base, an organic solvent, or heat on the enzyme leading to lose its structure and so its function.[8] This test depends on affecting enzyme activity as a function of changes in pH. In highly acidic media, the protein will be positively charged, which is attracted to the acid anions leading to protein precipitation and denaturation as a result of disrupting the salt bridges. [9,10] In addition, enzyme activity is compromised by sequestering its cofactor. Polyphenol oxidase is a copper enzyme i.e. the structure of the active site of the enzyme contains two copper ions. Adding of a chemical like phenylthiourea inhibit PPO by interact with copper ions at its active site.[11]

### Materials:

**Chemical**

Potato crude extract, 0.01M catechol, 5% TCA, phenylthiourea, distilled water.

**Equipment and Glassware**

Test tubes, rack, pipette, pipette pump, water bath.

### Protocol:

1. Label 3 test tube as **A**, **B** and **C**.
2. **In tube A (control)**: add 15 drops of the enzyme and 15 drops of catechol. Shake it.

3. **In tube B**: add 10 drops of the enzyme and 10 drops of TCA. Shake the tube thoroughly and after 5 minutes, add 10 drops of catechol.
4. **In tube C**: add 10 drops of the enzyme and few crystals of phenylthiourea. Shake the tube continually for 5 min, then add 10 drops of catechol.
5. Place all the tubes in the water bath at 37 ºC for 10 minutes.
6. Compare the results obtained from B and C to the control (A).

### Results:

| Tube | Degree of color intensity (Symbol: −, +, ++ or +++) |
|---|---|
| A (control) | |
| B | |
| C | |

## Experiment (4). Investigating the substrate specificity of polyphenol oxidase:

### Aim:

- To investigate the substrate specificity of the enzyme using structurally related chemicals.

### Principle:

Enzymes bind with chemical reactants called substrates. There may be one or more substrates for each type of enzyme, depending on the particular chemical reaction. The enzyme's active site binds to the substrate. Since enzymes are proteins, this site is composed of a unique combination of amino acid residues (side chains or R groups). The positions, sequences, structures, and properties of these residues create a very specific chemical environment within the active site.[3] A specific chemical substrate matches this site like a puzzle piece and makes the enzyme specific to its substrate. Potato polyphenol oxidase catalyses the oxidation of dihydroxy-and trihydroxy phenol to the corresponding quinine. [4]

### Materials:

**Chemical**

Potato crude extract, 0.01M catechol, 0.01 M phenol, 0.01M hydroquinone, distilled water.

**Equipment and Glassware**

Test tubes, rack, pipette, pipette pump, water bath.

### Protocol:

1. Label 3 test tube as **A**, **B** and **C**.
2. **In tube A (control)**: add 15 drops of the enzyme and 15 drops of catechol.
3. **In tube B**: add 15 drops of the enzyme and 15 drops of phenol.
4. **In tube C**: add 15 drops of the enzyme and 15 drops of hydroquinone.
5. Shake the tubes well.
6. Place all the tubes in the water bath at 37 ºC for 10 minutes. Shake each tube every 5 minutes to aerate, thereby adding oxygen to the solution.

### Results:

| Tube | Degree of color intensity (Symbol: −, +, ++ or +++) |
|---|---|
| A (control) | |
| B | |
| C | |

## Experiment (5). Investigating the effect of temperature on polyphenol oxidase activity:

### Aim:

- To investigate the effects of temperature on the enzyme activity.

### Principle:

Each enzyme has a temperature that it works optimally. Increasing temperature above 40 °C increases the rate of reaction, because it excites molecules and increases the rate at which enzymes/reactants collide and react to make product. However, increasing temperature too much may denature the enzyme and prevent it from working at all.[12] Meanwhile, at 0°C enzyme action is low because the movement of molecules is low. This causes the collision frequency between enzyme and substrate to be low.[13]

### Materials:

**Chemical**

Potato crude extract, 0.01M catechol, distilled water.

**Equipment and Glassware**

Test tubes, rack, pipette, pipette pump, water bath.

## Protocol:

1. Label 3 test tube as **A**, **B** and **C**.
2. **In tube A:** add 15 drops of the enzyme and incubate at 0 ºC for 10 min.
3. **In tube B**: add 15 drops of the enzyme and incubate at 37 ºC for 10 min.
4. **In tube C**: add 15 drops of the enzyme and incubate at 95 ºC for 10 min.
5. Add 15 drops of catechol for all tubes.
6. Shake the tubes well, the return the tubes to the proper temperature.
7. Wait for 15 minutes. Then, examine each tube without removing it from its temperature condition

## Results:

| Temperature (ºC) | Degree of color intensity (Symbol: −, +, ++ or +++) |
|---|---|
| 0 | |
| 37 | |
| 95 | |

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