Some factors affecting polyphenol oxidase activity
Poly phenol Oxidase Enzyme

- It is a copper-containing enzyme with an optimum pH of 6.7. It catalyses the oxidation of di- and tri-hydroxyl phenol to the corresponding quinone.

- This oxidation-reduction reaction is accompanied by a color change (quinones absorb light in the visible region of the spectrum).

\[
\text{Catechol} + \frac{1}{2} \text{O}_2 \xrightarrow{\text{polyphenoloxidase}} \text{o-quinone} + \text{H}_2\text{O}
\]
This reaction commonly occurs in nature and accounts for the "browning" of peeled potatoes and bruised fruits.

You will familiarize yourself with the reaction catalyzed by the enzyme polyphenol oxidase, as it occurs removed from the intact living cell, i.e. in a test tube.
Objectives:

1- To demonstrate *activity of the enzyme* polyphenol oxidase in crude extract prepared from potato.

2- To demonstrate the *chemical nature* of the enzyme.

3- To investigate the *substrate specificity* of the enzyme.

4- To investigate the *effects of various temperatures* on the activity of the enzyme.
This experiment is in four parts:

A) Enzyme Activity

B) Chemical Nature of polyphenol oxidase

C) Substrate Specificity

D) Temperature and Enzymatic Activity.
A) Enzyme Activity:

Occurrence of an enzymatic reaction which is the conversion of one molecule into another; a chemical reaction catalyzed at the active sites on the enzyme.

Method:

a) Label three clean test tubes A, B and C.
<table>
<thead>
<tr>
<th></th>
<th><strong>A1</strong></th>
<th><strong>B1</strong></th>
<th><strong>C1</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enzyme (15 drops)</td>
<td>Enzyme (15 drops)</td>
<td>Catechol (15 drops)</td>
</tr>
<tr>
<td></td>
<td>Catechol (15 drops)</td>
<td>distilled water (15 drops)</td>
<td>distilled water (15 drops)</td>
</tr>
</tbody>
</table>

Place the tube in a water bath at 37 °C.

Shake the tube every 5 min to aerate, thereby adding oxygen to the solution.

Every 5 minutes, after shaking, hold the tubes up to the light and examine. **Record the color** in each tube. Continue for 25 minutes.
All known enzymes are **proteins**. They are high molecular weight compounds made up principally of chains of **amino acids** linked together by **peptide bonds**.

Therefore it is expected that every factor effect on protein will effect the enzyme relatively.

These factors could be:

* Temperature, PH value, conc. of certain substances e.g. [S], [E] or [I].

**Method:**

a) Label four clean test tubes A, B, C and D.
<table>
<thead>
<tr>
<th></th>
<th>A2</th>
<th>B2</th>
<th>C2</th>
<th>D2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 drops of enzyme</td>
<td>10 drops of enzyme</td>
<td>10 drops of enzyme</td>
<td>15 drops of enzyme</td>
</tr>
<tr>
<td></td>
<td>15 drops of catechol</td>
<td>2ml of trypsin</td>
<td>10 drops of trichloroacetic acid</td>
<td>few crystals of phenylthiourea</td>
</tr>
<tr>
<td>Shake tube → place in water bath at 37 ºC for 10 min</td>
<td>Shake tube thoroughly → Place tube in a water bath at 37 ºC for 10 min</td>
<td>Shake tube thoroughly and wait 5 min</td>
<td>Shake tube thoroughly and continue shaking it frequently during a period of 5 min.</td>
<td></td>
</tr>
<tr>
<td>in the same water bath add 10 drops of catechol</td>
<td>in the same water bath add 10 drops of catechol</td>
<td>10 drops catechol</td>
<td>15 drops of catechol</td>
<td></td>
</tr>
<tr>
<td>Set tube aside as control with which to compare results of tubes B, C and D</td>
<td>Re place in the same water bath for 10 min. compare with tube A.</td>
<td>Place tube in water bath at 37 ºC for 10 min. compare with tube A.</td>
<td>Place tube in water bath at 37 ºC for 10 min. compare with tube A.</td>
<td></td>
</tr>
</tbody>
</table>
What is trypsin?

It is a protease found in the digestive system, where it hydrolyses proteins. It is produced in the pancreas as inactive proE (trypsinogen).

Trypsin cleaves peptide and used for many biotechnological processes.

The process is referred to as trypsin proteolysis or trypsinization, and proteins that have been digested/treated with trypsin are said to have been trypsinized.

How do you think it will effect the ppo activity?
Discussion

what is trichloroacetic acid?

It is an analogue of acetic acid widely used in biochemistry for precipitation of macromolecules, such as proteins, DNA, and RNA.

How do you think it will effect the ppo activity?
Discussion

phenylthiourea (PTU), has a very strong chemical affinity for the element copper. It is able to bind with copper, even when the copper is attached to other chemical substances, as in the active site of polyphenol oxidase.

How do you think it will effect the ppo activity?
PTU and catechol are poisons.
Avoid contact with solutions. Do not pipette any solutions by mouth.
Method:

a) Label three clean test tubes A, B and C.

<table>
<thead>
<tr>
<th></th>
<th>A&lt;sub&gt;3&lt;/sub&gt;</th>
<th>B&lt;sub&gt;3&lt;/sub&gt;</th>
<th>C&lt;sub&gt;3&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 drops of enzyme extract</td>
<td>15 drops of enzyme extract</td>
<td>15 drops of enzyme extract</td>
</tr>
<tr>
<td></td>
<td>15 drops of catechol solution</td>
<td>15 drops of phenol solution</td>
<td>15 drops of hydroquinone</td>
</tr>
</tbody>
</table>

Shake the tubes gently and place them in a water bath at 37 °C.

Examine the tubes after 5 min and 10 min. Record the color in each tube,
The three compounds used as substrates in this part of the experiment are structurally related, as shown in the figure below. Each is capable of reacting with oxygen to form various colored products.

Which tube do you think will show best result? And why?
The reaction rate increases with temperature to a maximum level, then suddenly declines with further increase of temperature. Because most animal enzymes rapidly become denatured at temperatures above 40°C.
### Method

a) Label three clean test tubes A, B and C.

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 drops of enzyme extract</td>
<td>15 drops of enzyme extract</td>
<td>15 drops of enzyme extract</td>
</tr>
<tr>
<td>Place the tube in a water: 0 °C for 10 min.</td>
<td>Place the tube in a water:37 °C for 10 min.</td>
<td>Place the tube in a water:70 °C for 10 min.</td>
</tr>
</tbody>
</table>

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In the same water bath add to each tube 15 drops of catechol

Shake the tube gently and quickly return it to its proper temperature condition.

Wait for 15 min. →examine the tube, without removing it from its temperature condition, and record the color in each tube
Which tube do you think will show best result? And why?
Thank You