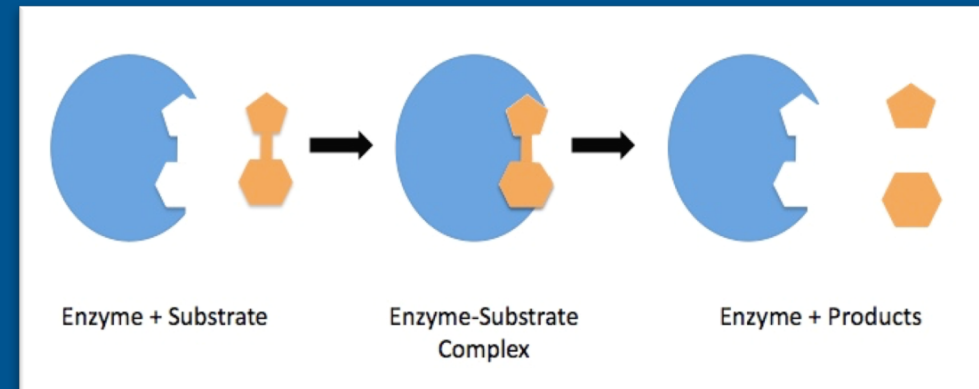
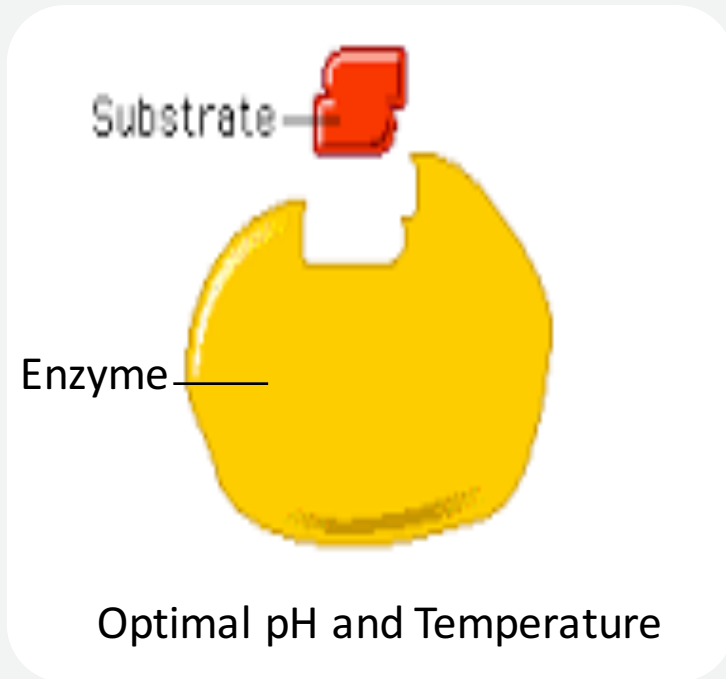


Introduction to Enzymology

-Some factors Affecting polyphenol Oxidase Activity



Important notes:



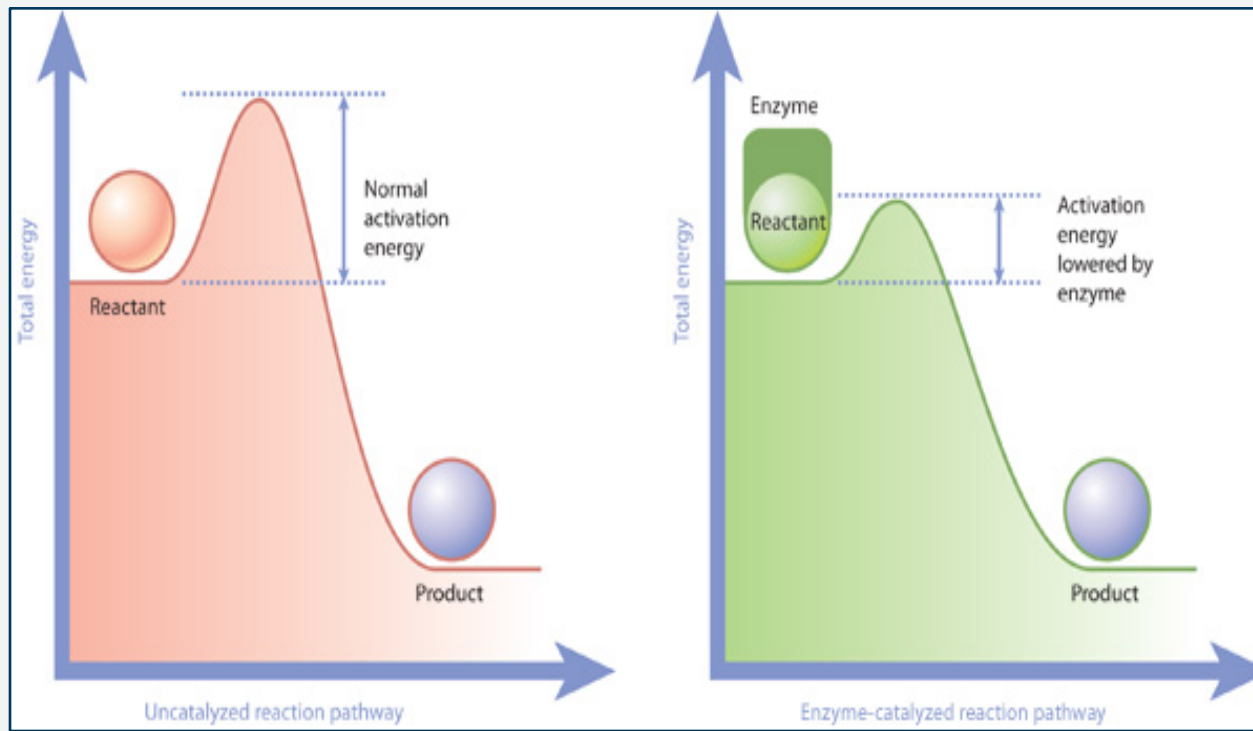
- There are 3 terms for an enzymatic reaction to happen:

1. Presence of enzyme
2. Presence of substrate
3. Availability of appropriate conditions (37 C, pH 6.7)

→ If any of these terms are absent there will be no enzymatic reaction.

How can enzyme increase the rate of a biochemical reactions?

1. Lowering the activation energy
2. Reducing the chance in the collisions of molecules or ions

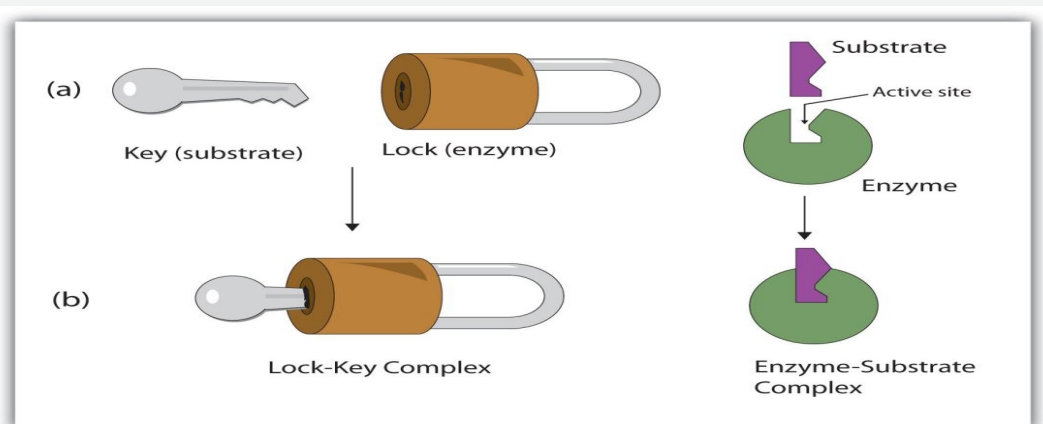


Activation energy : Is the energy that must be overcome in order for a chemical reaction to occur/the minimum energy required to start a chemical reaction ,given in units of kilojoules/mole.

How can substrate bind to the Enzyme?

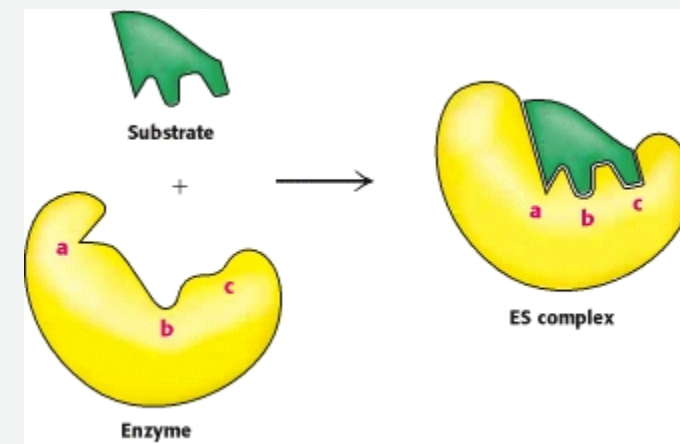
1- The Key and lock hypothesis

- Both the enzyme and the substrate possess specific complementary geometric shapes that fit exactly into one another




2- Induced fit model

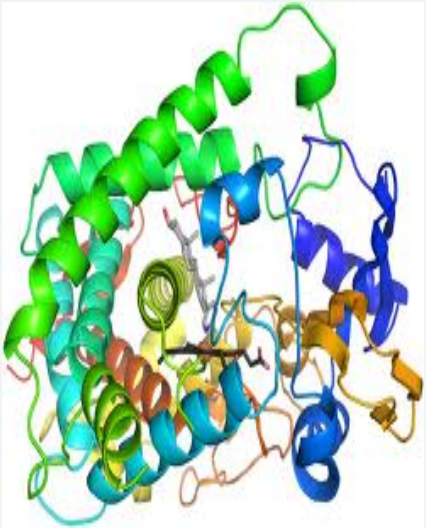
- The substrate does not simply bind to a rigid active site; the amino acid side chains which make up the active site are molded into the precise positions that enable the enzyme to perform its catalytic function.



Studying Enzymes

- **Because hundreds of reactions are** simultaneously carried out in the living cell, it becomes difficult to study a single reaction in an intact living cell.
 - **However,** it is possible to extract enzymes from cells and thus study enzyme catalyzed reactions in a test tube. In this experiment, a crude extract of the enzyme polyphenol oxidase will be prepared from the potato.
- 

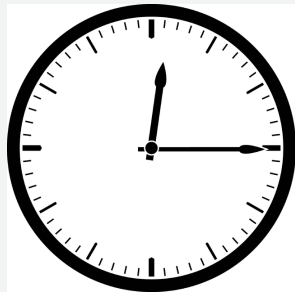
- **To detect and follow** the progress of the reaction in this experiment a simple, qualitative method will be used.
- **More sophisticated**, quantitative methods of following enzyme catalyzed reactions will be introduced later in the course.
- **In This Lab Polyphenol Oxidase** will be studied as an example to understand factors that could affect enzymes



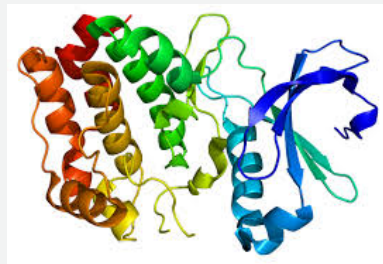
Objectives:

polyphenol oxidase in crude extract prepared from potato.

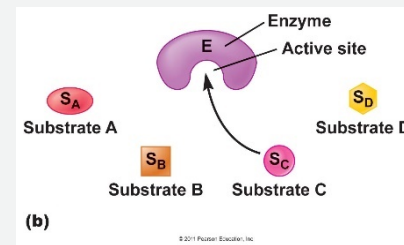
The effect of time



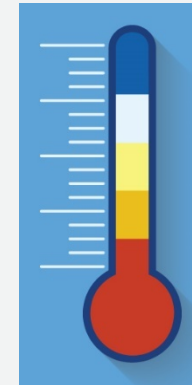
Chemical nature of this enzyme



Substrate Specificity



The effect of temperature



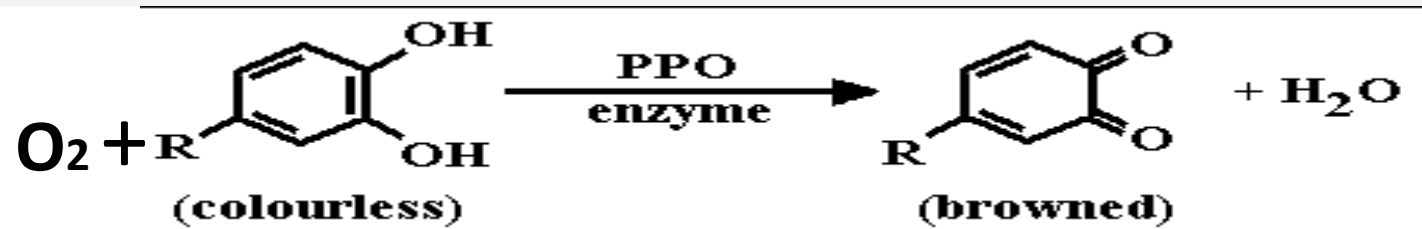
- In this experiment you will notice the change qualitatively (change in the color only)

Polyphenol Oxidase:



Enzymatic Browning

- **Polyphenol oxidase (PPO):**
- **Copper-containing enzyme** with an optimum pH of 6.7. It mainly catalyses the oxidation of **dihydroxy- and trihydroxy phenol** to the corresponding quinone.
- **This oxidation-reduction reaction** is accompanied by a color change
- **This reaction commonly** occurs in nature and accounts for the "browning" of peeled potatoes and bruised fruits.



1-Time course study of enzymatic Activity:

Principle:

- In this reaction we are looking for enzyme substrate reaction in general
- The intensity of this color (**brown**) will be proportional to the enzyme's activity in the tube under observation.
- You must notice the color change with the time.



1. *Time course study of enzymatic Activity:*

- a. Label three clean test tubes A, B and C.
- b. Prepare each tube as follows:

Tube	Addition
TUBE A	<ul style="list-style-type: none">• 15 drops of enzyme extract.• 15 drops of 0.01M catechol solution
TUBE B	<ul style="list-style-type: none">• 15 drops of enzyme extract.• 15 drops of distilled water.
TUBE C	<ul style="list-style-type: none">• 15 drops of 0.0M catechol solution.• 15 drops of distilled water

- c. Place all three tubes in a water bath at 37 °C.
 - d. Shake each tube every 5 minutes to aerate, thereby adding oxygen to the solution.
 - e. Every 5 minutes, after shaking, hold the tubes up to the light and examine.
-

Results:

1-Enzymic activity: Record the color in each tube, according to the scheme described in this page, in the table in the "Results" section. Continue for 25 minutes.

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

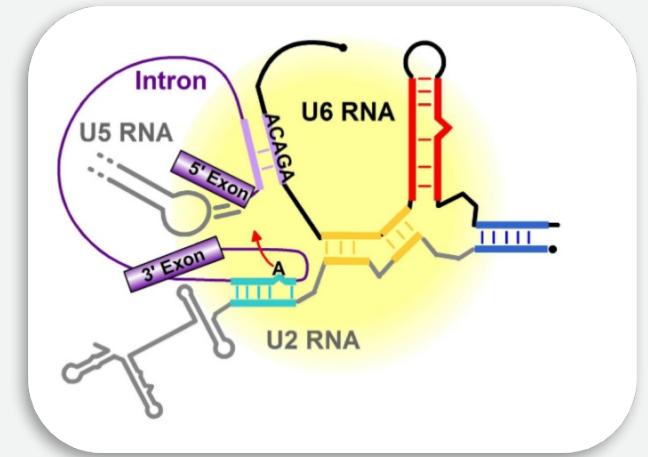
What do you noticed about the color at the end, is there any further change at 25 min?

2-CHEMICAL NATURE OF POLYPHENOL OXIDASE:

- **Majority of enzymes** are proteins. Some are made of RNA
- **The nature of polyphenol oxidase** will be examined, wither is it protein or not



Protein (Enzyme)



RNA(catalytic)

2. Chemical nature of polyphenol oxidase:

- a. Label four clean test tubes A, B, C and D.
- b. Prepare, and test, each tube as follows:

Tube	(1)	(2)	(3)
TUBE A	<ul style="list-style-type: none">• 15 drops of enzyme extract.• 15 drops of 0.01M catechol solution	<ul style="list-style-type: none">• Shake tube and place in water bath at 37 °C for 10 minutes.• As a Control!	

TUBE B	<ul style="list-style-type: none"> • Add 10 drops of enzyme extract. • Add 10 drops of 5% trichloroacetic acid. 	<ul style="list-style-type: none"> • Shake tube thoroughly and wait 5 minutes. 	Add 10 drops of 0.01M catechol solution. Place in water bath at 37 °C for 10 minutes
TUBE C	Add 15 drops of enzyme extract. Add a few crystals of phenylthiourea	Shake tube thoroughly and continue shaking it frequently during a period of 5 minutes.	Then add 15 drops of 0.01M catechol solution. Place in water bath at 37 °C for 10 minutes

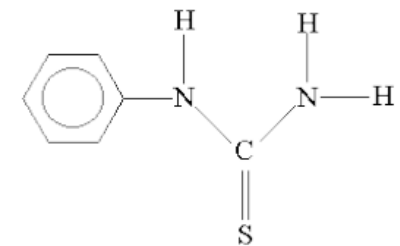
- Examine and compare with tube A.
- Record your observations in the table in the "Results" section.

Results:

2-Chemical nature of polyphenol oxidase

Tube	Treatment	Degree of color intensity (Symbol: -, +, ++ or +++)
A	Control	
B	TCA	
C	Phenylthiourea	

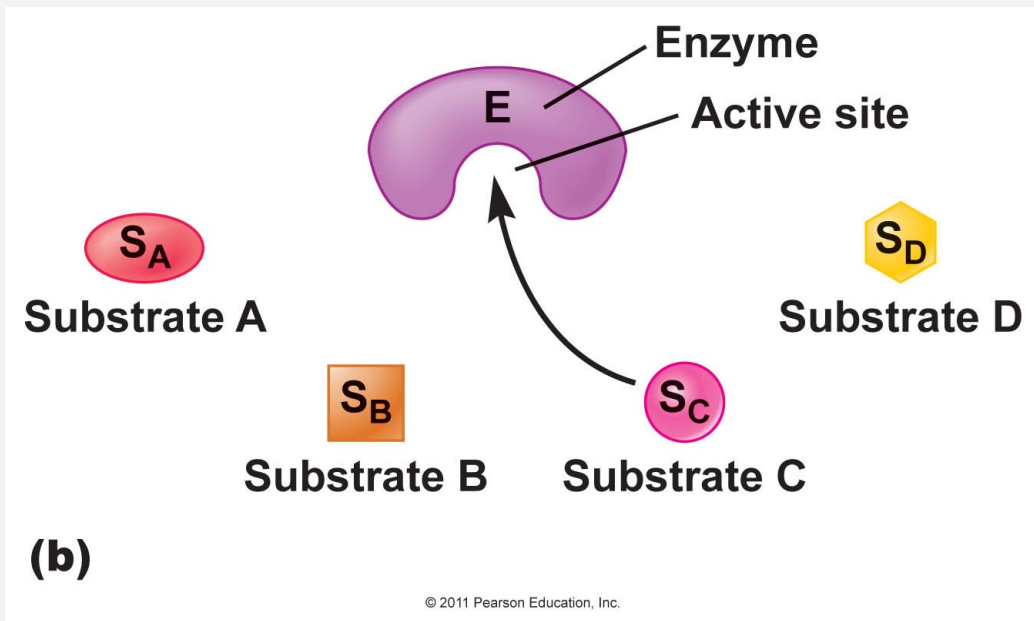
- **Trypsin:** a proteolytic enzyme
- **TCA:** an acid(Low pH) that precipitate and denature proteins
- **Phenylthiourea:** Phenylthiourea has a very strong chemical affinity for the element copper.



Phenylthiourea (PTU)

3- Substrate specificity:

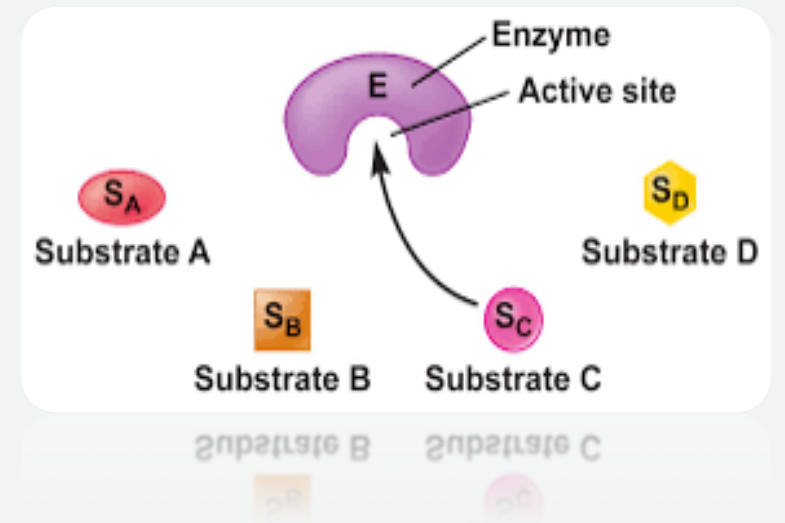
- The substrate binds to the enzyme at the active site. Since enzymes are proteins, this site is composed of a unique combination of amino acid residues (side chains or R groups). Which play a role in the specificity of the enzyme toward substrate



Types of Enzyme Specificity

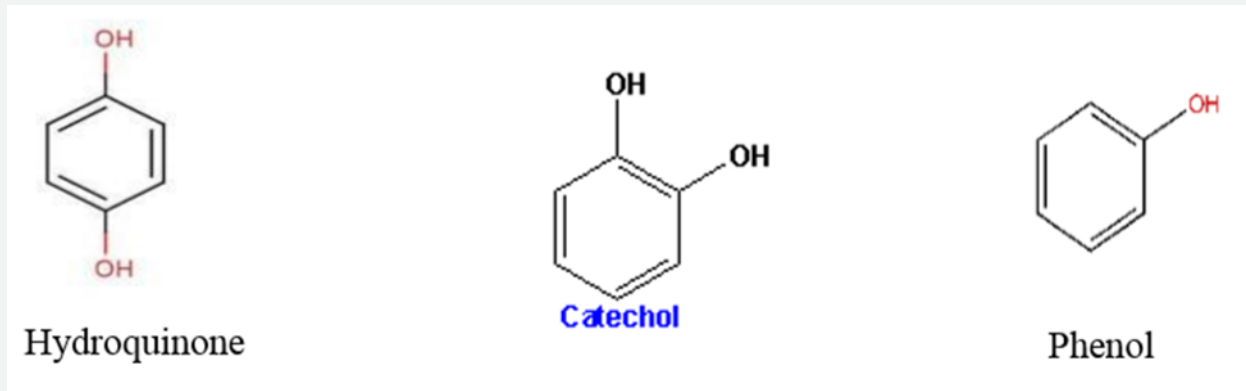
- There are four distinct types of specificity:

- ✓ **Absolute specificity** - the enzyme will catalyze only one reaction.
- ✓ **Group specificity** - the enzyme will act only on molecules that have specific functional groups, such as amino, phosphate and methyl groups.
- ✓ **Linkage specificity** - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure.
- ✓ **Stereochemical specificity** - the enzyme will act on a particular steric or optical isomer.



3-Substrate specificity:

- The three compounds (mono- and di-hydroxyl phenol) will be used to find out which one of them is a substrate for PPO



3-Substrate specificity:

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

Tube	(1)	(2)
TUBE A	<ul style="list-style-type: none">• 15 drops of enzyme extract.	<ul style="list-style-type: none">• Add 15 drops of 0.01M catechol solution
TUBE B	<ul style="list-style-type: none">• 15 drops of enzyme extract.•	<ul style="list-style-type: none">• Add 15 drops of 0.01M phenol solution
TUBE C	<ul style="list-style-type: none">• 15 drops of enzyme extract.	<ul style="list-style-type: none">• 15 drops of 0.01M hydroquinone

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

c. Examine the tubes after 5 minutes and after 10 minutes. Record the color in each tube, in the table in the "Results" section.

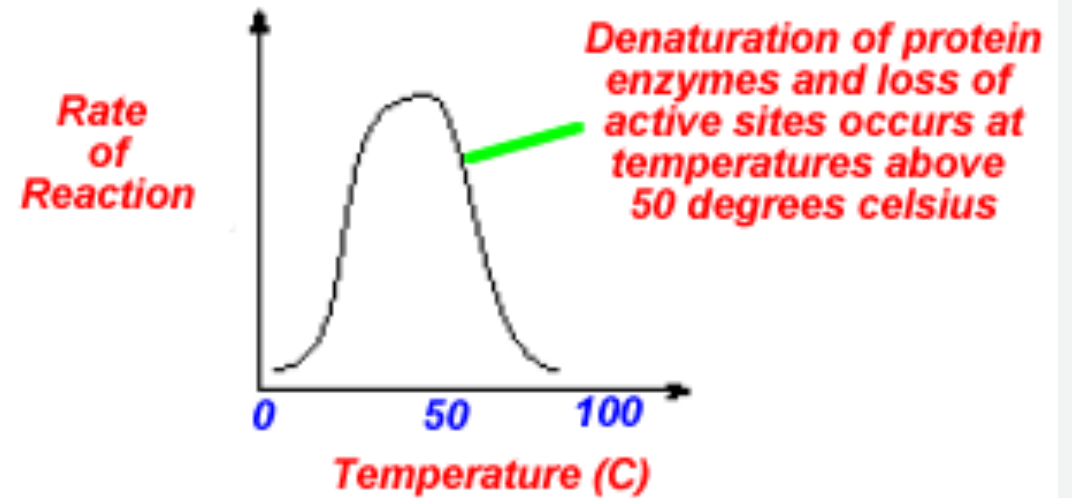
d. The three compounds used as substrates in this part of the experiment are structurally related, as shown in the figure below. Each is capable of reacting with oxygen to form various colored products.

- Results:
- Substrate specificity:

Substrate	Degree of color intensity (Symbol: -, +, ++ or +++)	
	5 minutes	10 minutes
Catechol		
Phenol		
Hydroquinone		

4-Temperature and enzymatic activity:

Enzyme Activity and Temperature



Optimum T_m :

is that T_m at which the activity of the enzyme is maximum

4-Temperature and enzymatic activity:

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

Tube	(1)	(2) Incubate At:	(3)
TUBE A	<ul style="list-style-type: none">Add 15 drops of enzyme extract to each tube.	0 °C Wait for 10 min	Add 15 drops of 0.01M catechol solution to each tube.
TUBE B	<ul style="list-style-type: none">Add 15 drops of enzyme extract to each tube.	37 °C.	Add 15 drops of 0.01M catechol solution to each tube.
TUBE C	<ul style="list-style-type: none">Add 15 drops of enzyme extract to each tube.	70 °C.	Add 15 drops of 0.01M catechol solution to each tube.

- b. Shake each tube gently and quickly return it to its proper temperature condition.
- c. Wait for 15 minutes. After this time, examine each tube, without removing it from its temperature condition, and record the color in each tube, according to the scheme described on page 3, in the table in the "Results" section.

4-Temperature and enzymatic activity

Temperature (°C)	Degree of color intensity (Symbol: -, +, ++ or +++)
0	
37	
70	

Discussion

- Discuss in each experiment what you observing and the reasons behind the findings

