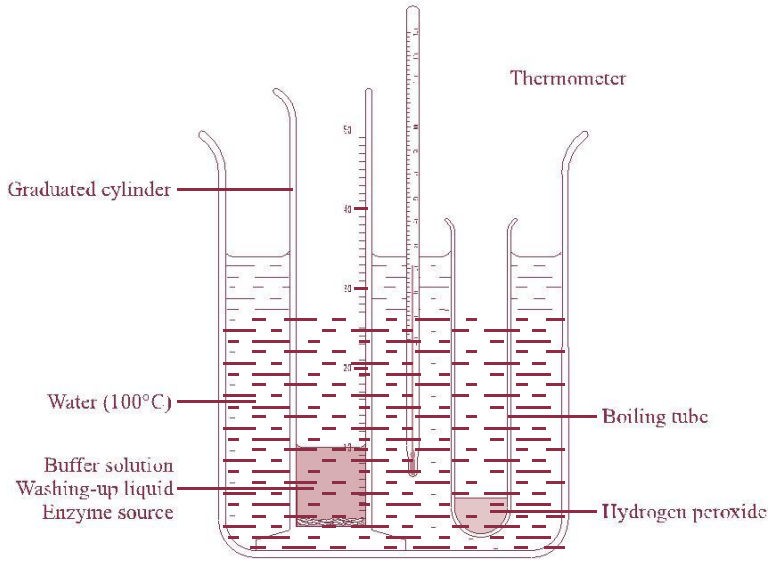
Investigate The Effect of Heat Denaturation On Catalase Activity

Biology – Leaving Cert

Experiments

Materials/Equipment

Enzyme source eg. Radishes	Knife
Hydrogen peroxide (20% or less)	Chopping board
Buffer solution (pH9)	Electronic balance
Boiling tubes	Weigh boats
Washing-up liquid	Dropper
Syringe	Thermometer
2 Water baths (25˚C, 100 C)	Disposable gloves
Graduated cylinders (100 cm³)	Timer
Test-tube holder	Test-tube rack



Procedure

1. Familiarise yourself with all procedures before starting.
2. Place 5 g of finely chopped radish into a boiling tube (without water) and place the boiling tube into the water bath at 100 °C for ten minutes.
3. Remove the boiling tube and allow it to cool.
4. Add 20 cm^3 of the buffer to the graduated cylinder.
5. Using the dropper, add one drop of washing-up liquid.
6. Add 5 g of the heated radish to the cylinder.
7. Add 2 cm^3 of hydrogen peroxide to the boiling tube.
8. Stand the cylinder and the boiling tube in the water bath until the desired temperature (25 °C) is reached.
9. Pour the hydrogen peroxide into the cylinder.
10. Note the presence or absence of foam formation and record.
11. Repeat the procedure from step 5 using an unheated radish sample.

Result

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Foam formation		

Conclusion/Comment

SKILL ATTAINMENT

INVESTIGATE THE EFFECT OF HEAT DENATURATION ON CATALASE ACTIVITY

Following instructions
Familiarise yourself with all procedures before starting
Follow instructions step by step
Listen to the teacher's instructions

Correct manipulation of apparatus
Prepare the enzyme source
Use the syringe
Use the graduated cylinder
Use the thermometer
Use the electronic balance
Set up and maintain the water baths
Use the test-tube holder
Use the timer

Observation
Observe the presence or absence of foam formation

Recording
Write up the procedure
Tabulate the results

Interpretation
Draw reasonable conclusions from your observations and results

Application
Become aware of any other application(s) of what you learned in this activity

Organisation
Exercise caution for your personal safety and for the safety of others Work in an organised and efficient manner
Label as appropriate
Work as part of a group or team
Clean up after the practical activity

Background information

An enzyme is a protein composed of a chain of amino acids, joined together by covalent bonds called peptide bonds. The actual activity of an enzyme is derived from the way this protein (polypeptide) chain is folded into a three dimensional structure (tertiary structure). When this three dimensional structure is destabilised by heat, typically at temperatures over 50 °C, denaturation is said to have occurred. This means that the linear sequence of amino acids unique to that protein (its primary structure) still exists but the enzyme has lost its activity. Enzymes catalyse reactions by forming a complex with the substrate (known as the enzyme-substrate complex) at a specific region of the enzyme called the active site. Denaturation occurs because the hydrogen bonds and the disulfide bonds which determine the tertiary structure of the enzyme, and consequently help to maintain the shape of the active site, are broken and do not reform with cooling. Thus enzyme-substrate complexes can no longer be formed and enzyme activity is lost.

Advance preparation

- Prepare the buffer solution.
- Buy fresh radishes.
- Set up the water baths and check the temperatures with a thermometer.

Helpful hints

- The enzyme source must be fresh.
- Besides radish there are many other good sources of catalase. Liver is the best source but celery and potato are also good. These can be used chopped, as described in the investigation, or extracts can be prepared. To prepare celery extract, chop three stalks and macerate them in a blender in 100 cm^3 of distilled water. Filter through a coffee filter. If using liver, macerate
  5 g in 100 cm^3 of distilled water and strain through a household sieve. Use 1 cm^3 of these extracts in each cylinder. Increase this volume if activity is low.
- Enzyme preparations lose activity very quickly. Therefore, the enzyme extract must be prepared immediately before use.
- The concentration of hydrogen peroxide may be expressed both as percentage and volume e.g. 30% (100 vol.).
- 6% (20 vol.) hydrogen peroxide is available in pharmacies. Other percentages are available from laboratory suppliers. Concentrations below 20% are irritants, concentrations above this are corrosive and cause burns.
- Hydrogen peroxide should be stored in a lightproof bottle because light accelerates its decomposition.
- Buffer tablets and capsules are available. Make these up with distilled water according to the instructions on the container. Liquid buffers can also be purchased.