

13. Investigation of the effect of pH on enzyme activities

Invertase (sucrase) catalyses the hydrolysis of sucrose to glucose and fructose. Its activity has been demonstrated in bacteria, moulds, higher plants and in gut of animals.

The optimum pH of invertase is between 4 and 6. The optimum pH of invertase from yeast is between 4.7 and 4.9, while that from honey is between 5.5 and 6.2. The maximum rate of hydrolysis by yeast invertase is obtained when the concentration of sucrose solution is 5-10%. Heavy metals such as Cu^{2+} , Hg^{2+} and Ag^{+} will also inhibit its activity.

In this investigation, the invertase is extracted from the gut of grasshopper. The rate of enzyme activity can be determined by estimating the amount of red/orange precipitate obtained on boiling with Benedict's solution.

Procedure

A. Extraction of invertase from grasshopper

1. Kill the grasshopper by decapitation. Remove its wings and limbs. Affix it with pins in a wax tray and cover it up with water or Insect Ringer. Dissection is done in water if it can be carried out quickly or in Insect Ringer if slowly.
2. Slit the grasshopper open dorsally and isolate the gut. Remove the thoracic muscles, fats and reproductive organs which cover the gut.
3. Transfer the gut onto a watch glass. Slit open the gut to remove any food or faecal content. Rinse with chilled distilled water.



Fig.1 Invertase

4. Place the gut in a mortar. Add 2 cm³ of chilled distilled water and grind it with a pestle. Allow the extract to settle.

B. The effect of pH on invertase activity

1. Prepare six test tubes each containing 1 cm³ of 10% sucrose solution. Label the tubes from 1A to 6A.
2. Add 1 cm³ of citrate-phosphate buffer solution at different pH values to the tubes as shown below.

Tube	1A	2A	3A	4A	5A	6A
pH of buffer solution	3.2	4.0	5.2	6.0	7.0	8.0

3. Label another 6 test tubes from 1B to 6B. Use a graduated pipette to add 3 cm³ of Benedict's solution to each tube.
4. Add 20 drops of enzyme extract to each "A" tube.
5. Allow the tubes to stand at room temperature for about 5 to 10 minutes.
6. Transfer the Benedict's solution in each "B" tube to the corresponding "A" tube. Shake the tubes gently and heat in a beaker of boiling water for 5 minutes. Measure the amount of red/orange precipitate formed.
(Students could be asked to design their own methods to assess the relative or absolute amount of red/orange precipitate formed.)

Note

1. The reaction time should be kept constant for all tubes.
2. Excess amounts of Benedict's solution should be used to ensure that all the reducing sugars formed are oxidized.
3. The amount of red/orange precipitate formed can be estimated or compared by one of the following methods:
 - Use an arbitrary system of '+' to denote the relative amount of precipitate.
 - Measure the depth of precipitate settled in the test tubes.
 - Weigh the precipitate formed. (The filter paper with the precipitate should be dried and cooled in a desiccator before weighing.)

Preparation of Reagent

1. Citrate-phosphate buffer

Buffers of different pH values are prepared by mixing solutions A (0.1M citric acid) and B (0.2M disodium hydrogenphosphate) in the proportions shown below.

pH	Volume of solution A (cm ³)	Volume of solution B (cm ³)
3.2	37.6	12.4
4.0	30.7	19.3
5.2	23.2	26.8
6.0	18.4	31.6
7.0	8.8	41.2
8.0	1.4	48.6