

## Investigating a Factor Affecting Enzyme Activity

### **INTRODUCTION:**

Enzymes are catalysts which speed up chemical reactions. The enzyme pectinase breaks down pectin present in the peel of fruits. This is a reason why it is used to increase the amount of juice extracted from fruits. Pectinase will act on its specific substrate, pectin. If temperature increases, random collision between substrate and enzyme will also get higher, so the rate of break down of polysaccharides to simpler substances will increase.

### **AIM:**

In this experiment I will investigate the effect of increasing temperature on the activity of the enzyme pectinase, that will be allowed to act on equal sized pieces of apples and the volume of juice compared.

### **HYPOTHESIS:**

Enzymes that are exposed to high temperatures, usually above 42°C, are denatured, which means their active site changes shape so no longer it can act on the specific substrate, causing it to lose its function as a catalyst. I expect my results to show this. At 5°C, little reaction will take place as the temperature is too low for the enzyme to act so a smaller volume of juice is expected. As the temperature increases to 15°C, the reaction would increase and at a temperature of 30°C, the rate of the reaction should have increased, so the volume of juice should be higher. At 40°C, the enzyme should be even more effective. The greatest volume of juice is expected here. At 65°C, the enzyme will have been denatured so little or no difference should be noticed.

### **APPARATUS:**

- water baths at 5°C, 15°C, 30°C, 40°C and 65°C
- pectinase solution at 2%
- 10 boiling tubes
- 2 graduated pipettes
- distilled water
- apples cubes of 1cm<sup>3</sup> each
- 1 tong
- 3 test tube racks
- thermostatically controlled water bath
- thermometer

- ice
- scalpel
- tile
- watch
- marker pen
- tweezer
- 25 ml measuring cylinder

### **VARIABLES:**

#### **Independent Variable:**

- **Temperature** – the effectiveness of the enzyme will be tested at 5°C, 15°C, 30°C, 40°C and 65°C. A beaker with ice and water will be used to ensure that the temperature of 5°C and 15°C will be used. For the other temperatures 3 water baths will be used.

#### **Controlled Variables:**

- **Precise timing for all experiments** – all test tubes will be left for 30 minutes in the water bath, keeping the time available for the enzyme to act the same. If one of them was removed before the full time designated, then the apple would be less affected compared to the rest and it would indicate that the enzyme activity was lower, leading to the wrong conclusion.
- **Sizes of the apples will be the same** – all apples will be cut in cubes of 1 cm<sup>3</sup>. This will keep the test with the enzyme at different temperatures fair. The surface area in contact with the enzyme should be made constant throughout the experiment.
- **PH of the substance** – all test tubes will only contain apple pieces and distilled water, so the pH for all will be the same.
- **Substrate concentration** – 2 equal sizes of apple cubes will be placed in each test tube, together with 7 ml of distilled water. Therefore, the amount of apple per test tube will be controlled and maintained fair for all experiments.
- **Volume of pectinase solution** – 2.5 ml of pectinase solution will be added to all the test tubes. By keeping this the same, the amount of enzyme will be equal for all cases, therefore allowing results to be fair. If more were placed in one of the test tubes, then the rate of activity of the enzyme at a

certain temperature would be wrongly judged, leading to incorrect conclusion.

- **Control** – For each temperature tested, apple cubes and distilled water will also be set.

#### **Dependent Variable:**

- The volume of juice extracted will be measured using a measuring cylinder.

#### **METHOD:**

1. The apparatus was collected.
2. Test tubes labelled: distilled water at different temperatures and pectinase at different temperatures.
3. 2 equal sized apple cubes ( $1\text{ cm}^3$ ) were placed in each boiling tube and were labelled according to the temperatures they would be placed into.
4. By using the dropper, 7 ml of distilled water was added to each of the boiling tubes.
5. Using the other dropper, 2.5 ml of pectinase was added to each of the boiling tubes.
6. The 5 boiling tubes were left for 30 minutes each: tube 1 at a temperature of  $5^{\circ}\text{C}$ , tube 2 at  $15^{\circ}\text{C}$ , tube 3 at  $30^{\circ}\text{C}$ , tube 4 at  $40^{\circ}\text{C}$  and finally tube 5 at  $65^{\circ}\text{C}$ .
7. Repeat the procedure using 9.5 ml of distilled water.
8. After 30 minutes, the tubes were collected using and placed inside the test tube rack to be observed.
9. The apples pieces were removed from each boiling tube with a tweezer and placed in a mortar.
10. The apple pieces were gently crushed.
11. The juice was collected and measured using the measuring cylinder.
12. The results were recorded.
13. The procedure was repeated for the apple cubes in each of the test tubes.
14. The volume of juices was compared.