**Chemistry Unit 4** Outcome 2

Food SAC Experiment

**Title**: Invertase in action.

**Aim**: To investigate the behaviour of the enzyme invertase

**Background**

Invertase (sucrase) is an enzyme that catalyses the hydrolysis of sucrose into fructose and glucose.



The progress of this reaction can be monitored through the use of the Benedict’s reagent. Benedict’s reagent produces a brownish colour in the presence of reducing sugar. Sucrose is not a reducing sugar but glucose and fructose are. Therefore a sucrose solution produces no colour change but a glucose or fructose solution will.

**Materials**

Yeast sachet

Water baths

Test tubes

* 1. M sucrose solution ( 9 g of table sugar to 250 mL tap water)

Benedict’s reagent

1. M NaOH

Buffer solutions: pH 1, pH 5, pH 7, pH 9, pH 11

**Invertase preparation**

Add a sachet of yeast to 175 mL of tap water in a flask.

Place in a 40 0C water bath for 30 minutes.

Centrifuge for 10 minutes.

Pipette off the supernatant liquid – this is the enzyme stock

**Part A: Enzyme functionality and pH**

Aim: To investigate the effect of pH on the activity of invertase



**Method**

A hot water bath set to 70 0C should be set up for the latter steps.

Add 2mL of each buffer to 5 separate test tubes. Label each test tube

Add 2 mL of sucrose and 2 mL of invertase to each tube. Leave for approximately 5 minutes.

Add 10 drops of 1.0 M NaOH solution to each test tube to establish an alkaline environment.

Add 2 mL of Benedict’s reagent to each test tube and add each test tube to the water bath

Time how long it takes for each test tube to reach a particular intensity of brown solution.

**Part B: Enzyme functionality and temperature**

**Aim**: To investigate the effect of temperature on the activity of invertase

**Method**

Set water baths at temperatures of 20 0C, 30 0C, 40 0C, 50 0C and 60 0C.

Place 2 mL of buffer 5 into each test tube and 2 mL of invertase.

Place one test tube in each water bath and leave 25 mins.

Add 2 mL of sucrose to each test tube.

Place back in the water bath in each test tube.

Take each test tube out and cool in an iced water bath (about 4 mins)

Add 2 mL of Benedict’s solution to each test tube.

Return the test tubes to the 70 0C water bath and time how long it takes for the brown colour to form.

**Results**

Appropriate presentation of results 4 marks

**Graphs**

For Part A draw a graph of time for brown formation vs pH of buffer.

For Part B draw a graph of time for brown formation vs pH of buffer. 6 marks

**Questions**

**Part A**

1. Put into your own words how Part A is designed to work. 3 marks

2. Identify the independent variable, the dependent variable and any controlled variables. 4 marks

3. What conclusion can you draw from your graph about the performance of invertase? 2 marks

4. Explain what is happening to invertase at a pH of 13. 2 marks

5. The equations below show the reactions occurring when Benedict’s reagent is added.

 a. What type of reaction is this?

 b. What is happening to the copper ions during this reaction?

 c. Why is there a colour change?

 d. Refer to the equations to explain why NaOH was added to the mixture. 5 marks



6. Glucose is a reducing sugar, sucrose is not. Glucose can form an open chain molecule with an aldehyde group. Sucose does not.

Explain what Benedict’s reagent is showing. 3 marks

**Part B**

7. Identify the independent variable, the dependent variable and any controlled variables. 4 marks

8. Discuss what your graph is showing about the impact of temperature on enzyme activity. 3 marks

9. a. Explain why the reaction rate increases from 20 0C to 40 0C.

 b. Explain why the reaction rate decreases from 40 0C to 60 0C. 4 marks

Teacher notes

The preparation of invertase is simple and a fresh batch leads to better results.

There is an assumption that the action of invertase stops when the NaOH is added to the test tubes.