SECTION B: BASIC BIOCHEMISTRY

At the end of this section, a student should be able to understand two main practical works:

1. Food test
2. Enzymology

1. FOOD TEST

Food test is the process of carrying out simple tests to identify different common food substances in their pure forms by using chemical reagents.

TYPES OF FOOD SUBSTANCES

During food test experiment the following types of food substances are expected to be identified:

- Reducing sugars
- Non-reducing sugars
- Starch
- Proteins
- Lipids

Each **A – level** practical examination of food test will usually consist of the following distribution of the total marks;

- Report writing ***(10 marks)***

Food tested	**Procedure**	**observation**	**inference**
Starch ½	½	½	½
Reducing sugars ½	½	½	½
Non-reducing Sugars ½	½	½	½
Protein ½	½	½	½
Lipid ½	½	½	½

- Any of the following theoretical questions should carry out 5 marks:

i. Identification of food substance(s).
ii. Natural sources of food substance(s).
iii. Roles/ function/ importance of food substance(s).
iv. Digestion pathway of food substance(s).
v. Storage form of food substance (in case of reducing sugar (*glucose*)).

vi. Deficiency disorder of food substance (in case of protein).

A. REDUCING SUGARS

Reducing sugars are carbohydrates which can reduce chemical reagents such as benedict solution during chemical reaction. Reducing sugars include all monosaccharides such as glucose, galactose and some disaccharides like maltose and lactose.

Natural sources:

Reducing sugars can be found naturally from the following sources:

i. Ripen fruits such as banana, mango, pawpaw and orange.
ii. Vegetables such as carrots and onion.
iii. Germinating cereals such as maize, millet, and sorghum.

Digestion pathway of reducing sugar:

Site of digestion	substrate	Enzyme	gland	product
Small intestine	maltose	maltase	Intestinal wall	glucose
Small intestine	lactose	lactase	Intestinal wall	Glucose and galactose

Storage form of reducing sugar (glucose):

Excess glucose is converted into glycogen under the influence of insulin hormone and stored in the liver or skeletal muscles in vertebrates.

Role of reducing sugars:

i. It is oxidized to yield energy during respiration process.

Test for reducing sugar:

Reagent

Benedict solution

Procedures:

Add 2cc of a solution containing reducing sugar in a test tube, then add 2cc of benedict solution and boil for about 5 minutes.

Observation:

The mixture shows a series of colour changes, the initial blue coloration turn green, then yellow, finally deep orange or brick red precipitates.

Basis of test

Benedict solution contains copper II sulphate (CUSO4), reducing sugars reduce soluble blue copper II sulphate containing copper II ions ($CU^{+)}$ to insoluble copper oxide containing copper (I) ions (CU^{+2}) which appears as brick red ppt.

B. NON REDUCING SUGARS

Non-reducing sugars are carbohydrates which cannot reduce chemical reagents such as benedict solution during chemical reaction. Non-Reducing sugars include sucrose only.

Natural sources:

Non -Reducing sugars can be found naturally from the sugar canes.

Digestion pathway of non-reducing sugar:

Site of digestion	substrate	Enzyme	gland	product
Small intestine	sucrose	sucrase	Intestinal wall	Glucose and fructose

Roles of non-reducing sugars:

i. It is oxidized to yield energy during respiration process.
ii. It is used as storage sugar in plant tuber such as sugar canes.

Test for non-reducing sugar:

Reagent

Benedict solution, dilute hydrochloric acid and sodium hydroxide.

Procedures:

Add 2cc of a solution containing non-reducing sugar in a test tube, then add 2cc of hydrochloric acid (HCL) and boil the mixture for around one minute, On cooling add 2cc of sodium hydroxide (NaOH) and finally add 3cc of benedict solution and boil the mixture again.

Observation:

The mixture shows a series of colour changes, the initial blue coloration turn green, then yellow, finally deep orange or brick red precipitates.

Basis of test

Boiling disaccharides in the presence of hydrochloric acid, help to hydrolyze it into monosaccharides which are reducing sugars. Therefore reducing sugars are formed; Benedict solution contains copper II sulphate (CUSO4), reducing sugars reduce soluble blue copper II sulphate containing copper II ions ($CU^{+)}$ to insoluble copper oxide containing copper (I) ions (CU^{+2}) which appears as brick red ppt.

C. STARCHES

Starches are polymers of α – glucose molecules which are linked together by 1, 4 and 1, 6 – glycosidic bonds.

Natural sources:

Starches can be found naturally in the following sources:

- Cassava
- Potatoes (sweet potatoes and Irish potatoes)
- Yams
- Cereals such as wheat, millet, sorghum, oats and maize.
- Unripen fruits.
- Ginger

Digestion pathway of starches:

Site of digestion	substrate	enzyme	gland	product
mouth	starch	Salivary amylase	Salivary gland	maltose
Duodenum	starch	Pancreatic amylase	pancreas	maltose
Ileum	maltose	maltase	Intestinal wall	Glucose molecules

Roles of starches:

i. It is oxidized to yield energy in the body during respiration process.
ii. It is used as the food storage material in the plant body.

Test for starch:

Reagent:

Iodine solution *(potassium iodide)*

Procedure:

Add 2cc of a solution containing starch in a test tube then add 2cc of iodine solution.

Observation:

Color change to blue black.

Basis of test:

The blue black formed is the result of the formation of the polyiodide starch complex.

D. PROTEINS

Proteins are organic compounds which contain carbon, hydrogen, oxygen and nitrogen; in some cases also contain sulphur (S) and phosphorous (P) elements.

Natural sources:

Proteins can be found naturally in the following sources:

- Eggs
- Meat
- Fish
- Peas and beans
- Milk

Digestion pathway of proteins:

Site of digestion	substrate	enzyme	gland	product
stomach	protein	Pepsin	Gastric gland	peptides
Duodenum	protein	Trypsin	pancreas	peptides
ileum	peptide	Erepsin	Intestinal wall	Amino acids

Storage form of proteins:

Excess proteins cannot be stored in animals, instead can be converted into urea and eliminated by the kidney as urine.

Deficiency disorders of proteins

Deficiency disorder of proteins may result into malnutrition disorder called ***kwashiorkor*** which is characterized by the following symptoms:

- Swelling of the legs, arms and stomach; a condition known as ***oedema***.
- Stunted growth.
- Hair progressively changes in texture and become soft.
- Skin lesions which cause a frankly paint appearance.
- Misery and apathy face known as moon face.

Roles of protein:

i. It is used for growth and repair the worn out body tissues, that is why proteins are very important in children and patient recovery from the operation and illness such as malaria.
ii. It provides body protection and defense against diseases.
iii. It is oxidized to yield energy when the body lacks carbohydrates and lipids.

Test for protein:

Reagent:

Reagent used as sodium hydroxide and copper sulphate.

Procedure:

Add 3cc of protein sample solution into a test tube, then add 3cc of sodium hydroxide solution, followed by 3cc drops of copper (II) sulphate solution and shake well the mixture.

Observation:
Colour of the mixture changes into purple colour.

Basis of test:
This test for peptide bond, whereby in the presence of dilute copper II sulphate in alkaline solution; nitrogen atoms in the peptide chain form purple complex with copper II ions.

E. **LIPIDS**
Lipids are organic compounds which contain carbon, hydrogen and oxygen but the proportion of oxygen is smaller than that of carbohydrates of the same molecular weight. The common lipids are fats, oils and waxes.

Natural sources:
Lipids can be found naturally in the following sources:

- Groundnuts
- Sunflower
- Milk
- Avocado
- Fats from animal meat

Digestion pathway of lipids:

Site of digestion	substrate	enzyme	gland	product
Duodenum	lipid	lipase	Pancreas	Fatty acids and glycerol

Roles of lipids:

i. It is oxidized to release energy during respiration process.
ii. It insulates the body against heat loss.
iii. It is a source of metabolic water.
iv. It forms structural components of the cell membrane.

Test for lipids:
Reagent
Reagent used is Sudan III solution *(red dye*).
Procedures:

Add 3cc of lipid solution into a test tube, then add 3 drops of Sudan III solution and shake the mixture gently and leave it to settle for around three minutes.

Observation:

A red stained oil layer (red ring) form on the surface of the clear solution.

Basis of test:

A red ring on the surface of the clear solution is due to stained fat globules which are stained red and are less dense than water.

FOOD TEST REPORT WRITING

Below are some important things to consider when writing a report for food test:

i. All the statement must be in the past tense to show that the experiment was already done; Example use "***WAS*** " and "***WERE*** " instead of "***IS*** "and "***ARE*** ".
ii. Specify the amount of food sample solution used; Example 3 drops of iodine solution instead of few drops of iodine solution.
iii. When testing for the sample solution and get negative results in the observation reports must retain the colour of the reagent; For example:
    - ***Brown colour*** of iodine solution.
    - ***Blue colour*** of benedict solution.
    - ***Blue colour*** of copper II sulphate.
    - ***Red colour*** of Sudan III solution.

iv. If the ***reducing sugar*** is present, non-reducing sugar is always present, but if reducing sugar is absent, test for non-reducing sugar.
v. When testing ***reducing sugars***, the color change may end up with yellow colour and not brick red due to low amount of reducing sugars present. Brick red is seen if there is a moderate amount of reducing sugar. In all cases wether the colour change ends up with yellow or brick red, report that reducing sugars were present.

vi. When your experiment involves more than one solution samples, the results will be tabulated on a single table by using the same procedures with two sample solution in separate test tubes, the same table on the observation and inference with two separate answer.

GENERAL PRACTICAL REPORT IN ONE SAMPLE SOLUTION (G)

FOOD	*PROCEDURES*	*OBSERVATION*	*INFERENCE*
Reducing	To 2cc of food sample	colour of the	Reducing

sugar	solution G, 2cc of benedict solution was added and the mixture were added and heated gently.	sample solution changed from blue to green to yellow to orange and finally to brick red ppt.	sugar was present in a sample solution G
Non-reducing sugar	To 2cc of food sample solution G in a test tube, 2cc of Hcl was added and the mixture was boiled and cooled. 2cc of NaOH was added to the mixture followed by 2cc of benedict solution then boiled again	colour of the sample solution changed from blue to green to yellow to orange and finally to brick red ppt.	Non-Reducing sugar was present in a sample solution G
Starch	To 2cc of food sample solution G in a test tube, 2 drops of iodine solution were added and the shaken gently	Color of the sample solution G changed into blue black	Starch was present
Protein	To 2cc of food sample solution G in a test tube, 2cc of NaOH was added, then 2 drops of COSO4 solution was added and shaken	Color of the solution G changed to purple	Protein was protein
Lipid	To 2cc of food sample solution G in a test tube, 2drops of Sudan III solution was added then shaken and allowed to settle for about 5 minutes.	A red stained layer was formed on the surface of the water	Lipid was present in the sample solution G

GENERAL PRACTICAL REPORT IN TWO SAMPLE SOLUTION G & M

FOOD	*PROCEDURES*	*OBSERVATION*	*INFERENCE*
Reducing sugar	To 2cc of food sample solution G and M in	A series of color changed from blue to yellow	Reducing sugar was present in a

	separate dry clean test tubes, 2cc of benedict solution was added and the mixture were added and heated gently.	and finally to brick red ppt in G while M retained blue colour of benedict	sample solution G but absent in M
Non-reducing sugar	To 2cc of food sample solution G and M in separate test tubes, 2cc of Hcl was added and the mixture was boiled and cooled. 2cc of NaOH was added to the mixture followed by 2cc of benedict solution then boiled again	A series of color changed from blue to yellow and finally to brick red ppt in G while M retained blue colour of benedict	Non-Reducing sugar was present in a sample solution G but absent in M
Starch	To 2cc of food sample solution G and M in separate test tube, 2 drops of iodine solution were added and the shaken gently	Color of the sample solution G changed into blue black while M retained the brown color of iodine	Starch was present in sample solution G but absent in M
Protein	To 2cc of food sample solution G and M in separate test tubes, 2cc of NaOH was added, then 2 drops of COSO4 solution was added and shaken	Colour of solution G changed to purple while M retained the blue colour of $CUSO_4$	Protein was protein in sample solution G but absent in M
Lipid	To 2cc of food sample solution G and M in separate test tube, 2drops of Sudan III solution was added then shaken and allowed to settle for about 5 minutes.	A red stained layer formed on the surface of the water in G while retained a clear red mixture	Lipid was present in the sample solution G but absent in M

Worked examples of food test

Worked example 1

PRE – NATIONAL MTWARA AND LINDI 2022, 3B

You have been provided with solution **L**, using the reagents provided carry out biochemical test to identify the food substances present on it:

a. Record your experimental work as shown in a table below:

FOOD TESTED	PROCEDURE	OBSERVATION	INFERENCE

b. i. Name the food substance (s) identified.
   ii. State the role(s) of the identified food substance to a person who has just recovered from malaria.
c. Which enzyme in the small intestine are involved in the digestion of the food substance identified.
d. State four adaptive features which enables the ileum to absorb digested food efficiently.

Solution:

a. Refer to the table of one sample solution (*reducing sugar and protein*).
b. i. Food substances identified are ***reducing sugar*** and ***protein***.
   ii. **Reducing sugar –** Provision of energy during respiration process.
   Protein – Repair the worn out body tissues.
c. Enzyme in intestine are:
   Erepsin – digestion of protein into amino acids.
   Maltase – digestion of maltose into glucose.
   Lactase – digestion of lactose into glucose and galactose.
d. Adaptive features of ileum:
   i. Presence of villi which increase area for food absorption.
   ii. Presence of many blood capillaries for transportation of food.
   iii. Presence of thin membrane for easy diffusion.
   iv. It is highly coiled for slow down movement of food.

Worked example – 02

DAR MOCK 2002; 3A

You are provided with sample solution **Z**, Design experiment to identify food substances present in the solution using chemical reagents provided.

a. Tabulate your experiment work in a table as shown below.

FOOD TESTED	PROCEDURE	OBSERVATION	INFERENCE

b. What is the role played by each food you have identified in your body?

c. Where do food substances in solution **Z** occur naturally?
d. Explain the deficiency disease and their symptoms due to lack of the food substances obtained from the solution.

Solution:

a. Refer to the table of one sample solution (*protein present).*
b. **Protein –** Repair the worn out body cells.
c. Protein can be found naturally in:
   i. Eggs
   ii. Milk
   iii. Beans
   iv. Meat
d. The deficiency diseases is called **kwarshikor** which is characterized by the following symptoms:
   i. Body swelling; a condition known as ***oedema***.
   ii. Stunted growth.
   iii. Hair progressively changes in texture and become soft.
   iv. Skin lesions which cause a frankly paint appearance.
   v. Misery and apathy face known as moon face.

Worked example – 03

NECTA 2002; 3A

The specimen B_1 is a mixture of different food substances. Design and carry out experiments to identify these foods using the reagents provided.

a. Record you're working as shown in the table below:

FOOD TESTED	PROCEDURE	OBSERVATION	INFERENCE

b. What role is played by each food substance you have identified in B_1 in children?
c. Excess of one of the food substance identified in B_1 is usually stored in the body:
   i. Name the hormone which influence the conversion of the food substance in the forms that can be stored in the body.
   ii. Write a word equation for the process in 2 (c) i above.
   iii. In which body organ does the process in 2 (c) I above occur?

Solution:

a. Refer to the table of one sample solution (*reducing sugar and protein)*
b. **Reducing sugar –** Provision of energy during respiration process.
   Protein – For growth and development
c. i. Insulin hormone.

ii. Glucose $\xrightarrow{\text{insulin hormone}}$ glycogen

iii. Liver and skeletal muscles.

Worked example 04
NECTA 2004, 3B

You have been provided with solution **A_4**.Perform an experiment using the reagents provided to identify the type of food substance (s) present in the solution.

a. Tabulate your results as shown below:

FOOD TESTED	PROCEDURE	OBSERVATION	INFERENCE

b. State one function of the food substance (s) identified in 1 (a).
c. For the food substance (s) identified in 1 (a), name one source in which each food substance can be obtained.
d. One of the food substance contained in **A_4** is important for a child growth and development:
   i. Identify the food substance.
   ii. State the parts of the alimentary canal where digestion of this food substance takes place.
   iii. In each part, name the enzymes involved in the digestion.

Solution:

a. Refer to the table of one sample (*starch, lipid, protein and Reducing Sugar).*
b. **Starch –** Provision of energy.
   Lipid – Insulation against heat loss.
   Protein – repair the worn out cells.
   Reducing sugar – Provision of energy during respiration process.
c. **Starch –** cassava
   Lipid – groundnut
   Protein – eggs
   Reducing sugar – onion

d. i. Protein
   ii. Stomach, duodenum and ileum.
   iii. Stomach – pepsin
   Duodenum – trypsin
   Ileum – Erepsin

Worked example – 05
MTWARA AND LINDI MOCK 2021; 3B

You have been provided with solution **A_2** and **A_3** which contains various food substances.

a. Use only chemical reagents provided to identify the food substance(s) present in solution **A_2 and A_3.** Tabulate your work as shown in the table below:

FOOD TESTED	PROCEDURE	OBSERVATION	INFERENCE

b. Name the food substance (s) identified in solution **A_2** and **A_3**.
c. Provide one (1) importance of the identified food to human body.
d. Give basis of the test of identified food in solution **A_3**.
e. Name the end products of digestion of the identified food.

Solution:

a. Refer to the table of two sample solution *(A_2 – protein and lipid) (A_3 – starch and protein).*
b. Importance of :
   i. **Protein –** Growth and development of the body.
   ii. **Starch –** Provide energy during respiration process.
   iii. **Lipid –** Acts as insulator against heat loss.
c. Basis of test of:
   Starch – blue black colour is the result of polyiodide starch complex.
   Protein – In the presence of dilute copper (II) sulphate (CUSO4) in alkaline solution Nitrogen atoms in peptide chain form purple complex copper (II) ions.
   Lipid - A red ring on the surface of the clear solution is due to stained fat globules which are stained red and are less dense than water.

d. End products of digestion:
   Starch - glucose
   Protein – amino acids
   Lipids – fatty acids and glycerol.

Worked example – 06
MTWARA AND LINDI MOCK 2021; 3A

You have been provided with solution **S_2** and S_3 which contain various food substances.

a. Use only chemical reagent provided to identify the food substance(s) present in solution S_2 and S_3. Tabulate your work as shown in the table below:

FOOD TESTED	PROCEDURE	OBSERVATION	INFERENCE

b. Identify the food substance (s) present in solution S_2 and S_3.
c. Outline two (2) functions of saliva.
d. Give reason to why sodium hydroxide *(NaOH)* solution was added after addition of dilute hydrochloric acid?
e. Excess of one food substance identified is NOT stored in the body:
   i. Name the food substance.
   ii. What is the fate of its excess in human body?

Solution:

a. S_2- Starch and non-reducing sugar whereby S_3 - protein and lipid**.**
b. Functions of saliva
   i. It contains salivary amylase which hydrolyzes starch into maltose.
   ii. It lubricates and dissolves food substances.
c. Sodium hydroxide (NaOH) was added to neutralize the acidic medium.
d. i. Protein.
   ii. Excess proteins can be converted into urea and eliminates as urine by the kidneys.

Worked example – 07
KAGERA MOCK 2019; 3A
You have been provided with solution Y and Z.

a. Using the chemicals and reagents provided, carry out the biochemical experiment to identify the food substance (s) contained in each solution Y and Z. Tabulate your results as shown below:

FOOD TESTED	PROCEDURE	OBSERVATION	INFERENCE

b. Some of the identified food substance in above can be used as structural materials in living organisms:
   i. Name any two food substances which are structural materials.
   ii. Specify the part(s) of the body of living organisms where the food substance identified in (b) (i) above are used as the structural materials.

c. One of the food substance identified in (a) above when lacking in the diets of children it leads to a deficiency disease.
   i. Name the food substance and the deficiency disease.
   ii. Give two signs/ symptoms shown by a child with a deficiency disease named in c (i) above.

Solution:

a. **Y** - Starch and lipid and **Z** – protein and lipid.
b. i. Protein and lipid
   ii. Protein – hair, nails, muscles and bones.
   Lipid – skin and nerve cells.
c. i. Protein – kwarshikor
   ii. Stunted growth and progressively hair changes.

PREPARATION OF EXTRACTS FOR FOOD TEST EXPERIMENT

It is the preparation of a solution from a solid food sample such as cassava root tuber, Irish potatoes stem tuber and ginger.

Steps followed in preparing the extracts:

1. Peel the solid food sample to remove the outer layer by using a knife.
2. Cut a solid peeled sample into small pieces by using a knife.
3. By using mortar and pestle grind the pieces to obtain fine particles.
4. Put the grinded specimen into a beaker.
5. Put 10cc of water to dilute the grinded specimen and still the mixture.
6. Filter the mixture by using a filter funnel to obtain sample solution.

Worked example – 08

FEZA BOYS 2019

You have been provided with solution B. You also have been provided with specimen C, D and E without any contamination, prepare solution C, D and E respectively.

a. List the steps you followed in preparing the extracts from each of the specimens C to E.
b. Design and carry out tests to identify carbohydrates present in solution B to E using the given reagents.
c. Write the basis for each test above.
d. Why was important to use dilute hydrochloric acid in your tests?

Solution:

B – Milk solution D – Ginger
C – Irish potatoes E - Onion

2. ENZYMOLOGY

Enzymology is the study of enzyme activities; it explains how enzymes work with respect to internal cellular condition. In this practical work on of the following questions would appear instead of food test and carriers – **15 marks**:

2.1 Action of salivary amylase on starch.

2.2 Action of catalase enzyme on hydrogen peroxide *(H_2O_2)*.

2.1 ACTION OF SALIVARY AMYLASE ON STARCH

Worked example 1

You have been provided with 2% of starch solution.

a. Label test tubes 1 – 5, rinse the mouth with clean water to remove food particles and chew a rubber band to stimulate the flow of saliva then collect saliva in test tube number 5.
b. Using a syringe, place 5cc of 2 % starch solution in test tube 1 – 4.
c. Draw – up 4cc of saliva, place 2cc of saliva in each test tube 2 and 3 and shake well the test tubes to mix the contents. Leave test tubes for 5 minutes.
d. After 5 minutes, add few drops of iodine solution to test tube 1 and 2.
e. By using syringe add 2cc of benedict solution to test tube 3 and 4 and place both test tubes in a water bath for five minutes.

Compare the final color in test tubes, then complete the table of results as shown below:

TEST TUBE	CONTENTS	TEST TUBE WITH	RESULTS	INTERPRETATION
1	2% starch solution	Iodine solution		
2	2% starch solution + saliva	Benedicts solution		
3	2% starch solution + saliva	Benedicts solution		
4	2% starch solution	Benedicts solution		

Questions

a. What normally happens when iodine was added to starch solution?
b. Test tube 2 contained *starch solution* at the beginning of experiment, how do you explain the reaction with iodine at the end of the experiment?

c. What food substance is benedict's solution test for?
d. Was this food present in test tube 3 and 4 at the beginning of experiment
e. What evidence you have to support your answer.
f. What evidence is there to suggest that this food substance is present in test tube 3 at the end of experiment?
g. What chemical change have taken place in test tube 2 and 3 after adding saliva, which would explain the results in test tubes after applying *iodine* test and benedict test?
h. What part could saliva have played in this chemical change?
i. Suggest the control of experiment which would help to support your answer to **h** above.

Answer:

a. When iodine was added to starch solution, *blue black color* was observed.
b. In test tube 2 the reaction at the end of the experiment, was retained the color of reagent (no reaction with iodine).
c. Food substance the benedict's solution was test for was reducing sugar.
d. Evidence to support the answer; there was no reaction with benedict's solution in test tube 3 at the beginning because salivary amylase was not already reacted with starch and convert it into maltose. In test tube 4 there was no reaction because there was no saliva containing salivary amylase which converts starch into maltose, hence solutions retained the color of reagent.
e. Evidence to show that this food substance was present at the end of experiment in test tube 3 was that; when placed in water bath there was series of color changes seen from blue, green, yellow, orange and finally brick red ppt.
f. The chemical reaction that took place in test tube 2 and 3 after adding saliva was hydrolysis reaction.
g. The control of experiment was test tube number 1.

Worked example – 02

LAKE ZONE 2018; 3A

You are provided with 2% of starch solution labeled S_1.

a. Label test tube 1 -5, rinse the mouth with clean water to remove food particles chew a rubber band to stimulate the flow of saliva than collect saliva in test tube number 5.
b. Using a syringe/ measuring cylinder place 5 cm^3 of 2% starch solution in test tube 1 – 4.
c. Draw up 4 cm^3 of saliva place 2cm^3 of saliva in each test tube 2 and 3 and shake well the test tubes to mix the contents leave the test tube for 5 minutes.
d. After 5 minutes add 2 drops of iodine solution to the test tube 1 and 2.

e. By using measuring cylinder add 2cm3 of Benedict's solution to the test tube 3 and 4 and place both test tubes in water bath for 5 minutes.
f. Compare the final colour in test tubes than complete the table result as shown below:

TEST TUBE	CONTENTS	TEST TUBE WITH	RESULTS	INTERPRETATION
1	2% starch solution	Iodine solution		
2	2% starch solution + saliva	Benedicts solution		
3	2% starch solution + saliva	Benedicts solution		
4	2% starch solution	Benedicts solution		

a. What was the aim of this experiment?
b. What was observed in the mixture present in the test tubes?
c. Name the chemical reaction that takes place in test tube 2 and 3 after adding saliva which would express the results in these test tubes after applying the iodine test and benedicts test?
d. What is the role of test tube 1 in the experiment?
e. State the role played by saliva in this experiment.
f. Write the word equation showing the chemical process taking place in test tube 3.

Solution:

a. The aim of the experiment was to investigate the action of S. amylase on starch.

b. **In test tube 1:**
On addition of iodine solution in this test tube 1 containing 2% starch solution the blue black colour was formed which show that starch was present in solution present in test tube 1.
In test tube 2:
On addition of iodine solution in test tube 2 containing 2% of starch solution and saliva the mixture retained the brown colour of iodine solution which show that starch was absent in test tube 2.
In test tube 3:
On addition of Benedict's solution in test tube 3 containing 2% starch solution and saliva the series of colour change was observed from blue to green to yellow to orange and finally into brick red which show that reducing sugar was present.
In test tube 4:

On addition of Benedict's solution in test tube 4 containing starch solution, the blue colour of Benedicts solution was retained which show that reducing sugar was absent.

c. The chemical reaction that takes place in test tube 2 and 3 is digestion of starch by hydrolysis process.
d. It acts as control experiment.
e. It provides an enzyme known as salivary amylase.
f. Starch $\xrightarrow{\text{Salivary amylase}}$ maltose

Worked example – 03

- Rinse your mouth with distilled water.
- Collect 5cc of saliva by chewing the rubber band provided.
- Dilute the saliva to obtain 20cc of saliva solution.
- You are provided with solution A, B and C with different substrate concentration.
- Take three test tubes labeled A, B and C and put 2cc of the solution A, B and C respectively.
- To each test tube put 2cc of saliva solution (DO NOT SHAKE), followed by Iodine solution at approximately the same time. Not the starting time observe in each test tube look clear white. Note the time for the completion in each test tube.

Questions:

i. What is the aim of this experiment?
ii. Why the solution changes in the test tube?
iii. What is the nature of the substrate tested? Why?
iv. Which solution in the test tubes changes early? Why
v. Which solution in the test tube change late? Why?
vi. Write the word equation for the reaction taking place in the test tube A, B and C.

Answer:

i. The aim of this experiment was to investigate the action of salivary amylase on different substrate concentration.
ii. The solution changes in a test tube because *salivary amylase* hydrolyses starch into maltose which is clear colour.
iii. The nature of the substrate tested was starch. This is due to the fact that on addition of iodine solution; the solution turned into blue black due to the formation of polyiodide starch complex which appears blue black in colour.
iv. The solution in the test tube A changed fast (early), this suggest that in a solution A there was low substrate concentration and the reaction completed more readily due to unsaturation of salivary amylase active sites.

v. The solution in the test tube C changed slowly (late), this suggest that in solution C there was high substrate concentration. At high substrate concentration the reaction completes more slowly or tends to remain approximately less or more constant because all enzymes active site become fully saturated.
Table of results:

Test tube	Time(min)	Rate (min^{-1})
A	8	1/8 (0.125)
B	16	1/16 (0.0625)
C	32	1/32(0.03125)

vi. The word equation for the reaction taking places in test tube A, B and C is:

$$\text{Starch} \xrightarrow{\textit{Salivary amylase}} \text{maltose}$$

Worked example – 04

- Rinse your mouth by distilled water to remove food particles.
- Collect 5cc of saliva by chewing the rubber band provided.
- Dilute the saliva with distilled water to make 10cc of saliva solution.
- Take two test tube name them M and K.
- Add 4 drops of iodine solution in each test tube containing solution L provided and shake the mixture solution.
- At approximately the same time put 2cc of saliva solution in each test tube M and K, then in test tube K put 2cc of sodium chloride shake the mixture immediately after putting saliva solution and start the time and observe the colour changes until it changes into clear white in each test tube.

Questions:

i. What is the aim of this experiment?
ii. What is the nature of the substrate tested?
iii. Why is the solution change to clear white?
iv. Which solution change early? Why?
v. Which solution changes late? Why?
vi. What is the role of sodium chloride in test tube M and K.?
vii. Sketch the curve of M and K.

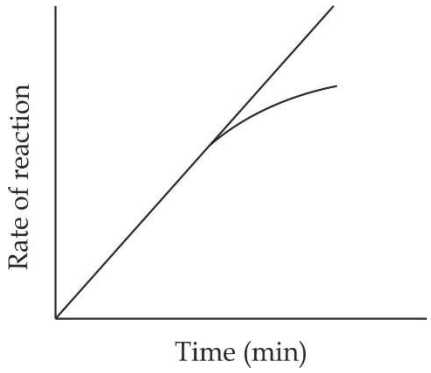
Solution:

i. The aim of this experiment was to investigate the action of salivary amylase on starch.
ii. The nature of the substrate tested was starch, this is due to the fact the solution provided (*solution L*) in test tube turned blue black on addition of iodine solution, iodine reacts with starch to form polyiodide starch complex which appears blue black in colour.

iii. The solution turn to clear white due to hydrolysis of starch by salivary amylase into colorless maltose (strong reducing sugar) which has no reaction with iodine solution.
iv. The solution in test tube K changes early due to the fact that sodium chloride solution so added yields chloride ions (CL^-) which activates hydrolysis of starch by salivary amylase, the reaction complete faster.
v. The solution in test M changes slowly since there was no activator of salivary amylase.
vi. The role of sodium chloride (Nacl) in this experiment was to provide chloride (CL^-) which are activators of the active site of *salivary amylase*.
vii. The word equation for this experiment is :

$$\text{Starch} \xrightarrow{\textit{Salivary amylase}} \text{maltose}$$

viii. The graph of the reaction for test tube K and M against time:



2.2 ACTION OF CATALASE ENZYME ON HYDROGEN PEROXIDE

Example - 01

You have been provided with 2% of hydrogen peroxide (H_2O_2) solution and some fresh liver tissues. Carry out the following experiment to investigate the reaction of substance X present in the liver tissue with hydrogen peroxide.

a. i. Label three test tubes 1. 2 and 3 place them on the test tube rack.
ii. Cut three cubes (each $1cm^3$) from the liver tissues provided.
iii. Put one of the cubes in the test tube.
iv. Grind the two cubes left in a mortar containing small amount of fine sands.
v. Divide the grinded cubes into two equal portions, put the first portions. Put the first portion into the test tube 2.

vi. Put the remaining portion of the grinded liver into the test tube 3 boil it with some little water and allow it to cool on the test tube rack.

vii. Add 4cm3 of hydrogen peroxide in test tube 1 and make accurate observation including the temperature of the test tube contents as shown in the table below.

viii. Repeat separately the procedure in the (vii) above with test tube 2 and 3 each time make accurate observation of your experiment, including the temperature and amount of gas produced.

Test tube	Temperature/ ^{o} C	Observation
1		
2		
3		

i. i. Name the substance X.

ii. Name the sub cellular units in which substance X occurs.

iii. Name the biological process catalyzed by X which takes place in the liver.

b. Suggest the purpose of:

i. Grinding the liver tissues with fine sands.

ii. Boiling the grinded liver tissue.

c. i. Write balanced equation between substance X and H_2O_2.

ii. What is the biological significance of substance X and H_2O_2?

iii. Name the gas produced by the reaction.

d. i. From your views and experimental observations regarding the rxn energetic, name the type of reaction shown between X and H_2O_2.

ii. Suggest the industrial use of substance X.

iii. Suggest the aim of the experiment.

Solution:

a.

Test tube	**Temperature/ ^{o} C**	**Observation**
1	20^{o}C	Less effervescence
2	20^{o} C	More effervescence
3	20^{o}C	No effervescence

b. i. Substance X was catalase.

ii. The subcellular unit in which substance X occurs is peroxisome.

iii. The biological process which is catalyzed by X in the liver is detoxification.

c. i. The purpose of grinding the liver with fine sands was for reduction of the mass which leads to increase in the *surface area* of the liver.

ii. The purpose of boiling the liver tissues was to denature the active sites of catalase as it was confirmed by observing no effervescence on addition of hydrogen peroxide into test tube 3.

d. i. The balanced equation between X and H_2O_2.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

ii. In living organisms most of organs and cells produce poisonous substances from their metabolic activities which when allowed to accumulate may lead into death of such organism. Some vital organs such as liver contain special subcellular organelles such as *peroxisome* that produce catalase which is essential for detoxification of harmful into harmless such as water and gas.

iii. Oxygen was the gas produced from the reaction.

e. i. The type of reaction from energetic study is exothermic reaction. Since it involved the liberation of temperature to the surrounding.

ii. Possible industrial uses of X:

- Synthesis of artificial antibiotics.
- Synthesis of antivenom.
- Synthesis of artificial immune.

iii. The aim of the experiment was to investigate the effect of the temperature on enzyme controlled reaction.

Worked example – 02

You are provided with a 2% of hydrogen peroxide (H_2O_2) solution and manganese (IV) oxide, liver and wooden splints. Carry out the following activities to investigate the effect of manganese (IV) oxide and liver on the hydrogen peroxide (H_2O_2).

a. Label four test tubes 1, 2, 3 and 4.
b. To test tube 1 add a spatula full of unheated manganese (IV) oxide.
c. To test tube 2 add a spatula of manganese (IV) oxide, heat for a minute and cool it.
d. Crush two small pieces of unboiled liver and place them into test tube 3.
e. Cut off other two small pieces of liver and place them into test tube 4. Add about 10cm^3 of water, then boil for about five minutes, cool it and pour water out of the test tube 4, crush the boiled liver using mortar and pestle then place the crushed liver into the test tube 4.
f. To test tube 1 add 2cm^3 of hydrogen peroxide (H_2O_2), and immediately cork the test tube, observe what happen to the mixture for about 1 minute then insert a glowing splint in the test tube.
g. Repeat the activities in (f) above for the remaining test tube 2, 3 and 4.
h. Record your observation in (f) as shown in the table below.

Test tube	Observation
1	

2	
3	
4	

Questions:

a. What was the aim of this experiment?
b. What chemical substance reacted with hydrogen peroxide when you use unboiled liver?
c. Write the balanced chemical equation for this reaction referred in (b) above.
d. What was the nature of the gas evolved during your experiment? Give reason for your answer.
e. What was the purpose of boiling liver in test tube 4?
f. What was the purpose of grinding liver in the test tube 3?
g. What is the biological significance of the concept being investigated in the experiment?

Solution:

Test tube	Observation
1	Lots of effervescence, glowing splint was relighted
2	Lots of effervescence, glowing splint was relighted
3	No effervescence, glowing splint was relighted
4	No effervescence, glowing splint was relighted

a. The aim of the experiment was to investigate the effect of temperature in inorganic catalyst (manganese (IV) oxide) and organic catalyst (catalase).
b. Catalase was a chemical substance reacted with hydrogen peroxide when unboiled liver was used.
c. The balanced equation between X and H_2O_2.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

d. The nature of gas evolved was oxygen, it relighted glowing wooden splint.
e. The purpose of grinding liver in test tube 3 was to increase surface area for the reaction to occur.
f. The purpose of boiling liver in the test tube 4 was to destroy (denature) the action of catalase enzyme.
g. Removal of toxic substances which would damage the lining cells.