EXPERIMENT a

Aim

To test for the presence of carbohydrates like glucose (sugar), sucrose and starch.

THEORY

Carbohydrates are the main source of energy in our body. The presence or absence of free aldehyde group makes them reducing or non-reducing sugars. Reducing sugars (mostly monosaccharides) give a positive response for Benedict's and Fehling's reagents in which blue colour of the reagent is changed to yellow a brownish red precipitate, depending on the concentration of sugar in the given sample.

MATERIAL REQUIRED

Test tubes, funnel, beakers, water bath, test tube holder, test tube stand, droppers, spirit lamp, reagents (Benedict's solution and Fehling's solutions A and B), concentrated HCl, saturated solution of NaOH, dilute Iodine solution, fruit juices of apple, grape or banana, extracts of potato, sugarcane or rice, etc. as sources of carbohydrate.

PROCEDURE AND OBSERVATIONS

1. Take freshly prepared juices of apples/grapes/banana to detect the presence of glucose and take sugarcane juice to detect the presence of sucrose in different test tubes.
2. Take extract of potato or rice to detect the presence of starch in another test tube.
3. Perform the following tests for detecting the presence of glucose, sucrose and starch and verify your results from the table given below.

1. TEST FOR GLUCOSE (monosaccharide)			
S.No.	**Test**	**Observation**	**Result**
(i)	**Benedict's Test**		
	(a) Take 2 ml of test solution (apple/ grape/banana juice) in test tube + 2 ml of Benedict's solution. **(b)** Boil over spirit lamp.	The colour changes initially from blue to green and finally to orange or brick red,	Reducing sugar, i.e. glucose is present.
(ii)	**Fehling's Test**		
	(a) Take 2 ml of test solution in test tube + 2 ml of Fehling's solution A+ 2 ml of Fehling's solution B. **(b)** Place the test tube in boiling water in a water bath.	The initial blue colour turns green to yellow and finally, a brick red or orange ppt. is formed.	Glucose (reducing sugar) is present.

OBSERVATION TABLE

Benedict's Test

S.No.	Name of the food items	Colour of the precipitate	Inference drawn
1.	Apple Juice		
2.	Banana Extract		
3.	Grapes Juice		
4.	Onion Juice		
5.	Milk		

Fehling's Test

S.No.	Name of the food items	Colour of the precipitate	Inference drawn
1.	Apple Juice		
2.	Banana Extract		
3.	Grapes Juice		
4.	Onion Juice		
5.	Milk		

2. TEST FOR SUCROSE (disaccharide)

S.No.	Test	Observation	Result
(i)	Perform Benedict's and Fehling's test with sugarcane extract.	No change in colour.	Sucrose present.
	Hydrolysis of sugar for carrying out sugar test		
	(a) Take 2 ml of sugarcane extract and add 2-3 drops of concentrated Hcl. **(b)** Boil the test tube gently for 2 minutes. **(c)** This will result in the hydrolysis of sucrose into glucose and fructose. **(d)** After cooling, add 4 ml of saturated solution of NaOH or Na_2CO_3, to neutralise the solution and divide the solution into two parts in two different test tubes (A and B).	The colour changes initially from blue to green and finally to orange or brick red,	Reducing sugar, i.e. glucose is present.
(ii)	**Benedict's Test and Fehling's Test**		

	(a) Perform Benedict's test in test tube A containing hydrolysed sample. **(b)** Perform Fehling's test in test tube B containing hydrolysed sample.	Monosaccharides (reducing sugar) are present in the samples.	

3. TEST FOR STARCH (polysaccharide)

S.No.	Test	Observation	Result
(i)	**Iodine Test**		
	(a) Take the extract of potato or rice (2 ml) in a test tube and add few drops of Iodine solution in it.	Blue-black colour is observed.	Starch is present in the sample.
	Hydrolysis of sugar stand solution I5, carrying out sugar tests.		
	(a) Take 2 ml of potato/rice extract + 2-3 drops of HCl. **(b)** Boil the test tube gently for 2 minutes. **(c)** After cooling, add 2-3 drops of NaOH to neutralise the solution.		
(ii)	**Benedict's Test and Fehling's Test**		
	Now, perform the Benedict's and Fehling's test with the above hydrolysed sample.	Change in colour from blue to orange or brick- red ppt. is observed.	Monosaccharides are present in the sample.

PRECAUTIONS

1. Use separate droppers for adding different reagents.
2. Proper care should be taken, while heating the samples in test tube.
3. Keep the mouth of test tube away from yourself and your friends.
4. Direct heating of test tube should be avoided. The heating should be carried out in water bath.
5. A suitable control by taking water instead of biological material should be run to compare the results.

VIVA VOCE

Q1. Name one reducing and one non-reducing sugar.

Ans. Glucose is reducing sugar and sucrose is non-reducing sugar.

Q2. What chemical structure makes a sugar reducing or non-reducing sugar.

Ans. Presence of free aldehyde group.

Q3. Name a disaccharide, which is a non-reducing sugar.

Ans. Sucrose.

Q4. Name few food stuff, which can be taken for the test of sugars.

Ans. Banana, grape juice, onion, rice, potato etc.

Q5. Name two reagents used for testing sugars.

Ans. Benedict's and Fehling's reagents.

Q6. Sucrose solution gives a negative result with Benedict's test. Why?

Ans. Sucrose (a disaccharide) does not contain the free aldehyde or ketone group and is unable to reduce the copper sulphate cupric ion (Cu^{2+}) into cuprous ion (Cu^{+}) of Benedict's solution, therefore, it gives negative result with Benedict's solution. But when it is hydrolysed, it breaks into smaller subunits (monosaccharide) and gives positive result for Benedict's solution.

Q7. Why are monosaccharides called reducing sugars?

Ans. Monosaccharides are the simplest carbohydrates that contain free aldehyde or ketone group and can convert cupric (Cu^{2+}) ions to cuprous (Cu^{+}) ions, e.g. glucose and fructose. Therefore, these are called as reducing sugars.

Q8. Give the common names for following sugars:

(i) Sucrose

(ii) Maltose

(iii) Lactose

Ans. The other name of the following sugars are as follow:

(i) Sucrose – Cane sugar

(ii) Maltose – Malt suga

(iii) Lactose – Milk sugar

Q9. Give the name of the sweetest naturally occurring monosaccharide.

Ans. Fructose or fruit sugar is the sweetest naturally occurring sugar found in honey and fruits.

Q10. What is the use of HCl in the test of carbohydrates?

Ans. Sucrose or starch are non-reducing sugars (disaccharide and polysaccharide, respectively) and when they are boiled with HCl, they are converted into their respective monosaccharides or reducing sugars. These monosaccharide units react with different reagents to prove the presence of carbohydrates in the sample extract.

Q11. Why does the blue colour disappear on boiling and reappear on cooling in the iodine test for starch?

Ans. Starch is actually a mixture of two components, i.e. water soluble amylose and water insoluble amylopectin. Amylose is a linear chain polymer which forms crystalline structure in the starch. When amylose comes in contact with iodine molecule, it gives the characteristic blue colour. When the solution of iodine with starch is heated the crystalline structure gets disrupted forming a random chain and the blue colour disappears. When it is cooled again amylose refolds into its crystalline form and the blue colour reappears.

Q12. Will iodine test give a positive result with glucose, fructose or sucrose solution?

Ans. No, iodine test will not give a positive result with glucose, fructose or sucrose solution because iodine specifically makes a blue coloured complex with starch. This blue-coloured substance may be an adsorption complex of starch with iodine rather than a definite compound.

EXPERIMENT b

Aim

To test the presence of proteins in suitable plant and animal materials.

THEORY

Proteins are the linear polymers of amino acids that are held together by peptide bonds A peptide bond joins the amino and the carboxyl group of successive amino acids. The presence of protein can be tested by biuret test, xanthoproteic test and Millon's test. Proteins contain nitrogen atom in the peptide chain, which forma violet coloured complex with copper ions in Biuret test. Xanthoproteic test is performed for the proteins containing aromatic amino acids. In this test, the benzene ring in the amino acids is nitrated by heating with nitric acid. It forms a nitro-complex (picric acid), which is yellow in colour and turns to orange when treated with an alkali.

MATERIAL REQUIRED

Test tubes, spirit lamp, test tube holder, test tube stand, droppers, mortar and pestle, filter paper, funnel, 40% NaOH, 1% $CuSO_4$, solution, concentrated HNO_3, 20% NaOH solution. Millon's reagent and milk or albumin of egg or gram seed (protein source).

PROCEDURE AND OBSERVATIONS

1. Take albumin of egg in a test tube or crush the gram seeds in mortar and pestle and transfer this crushed material in a test tube and boil. Then filter it in another test tube and use this filtrate as test solution.
2. Test for the presence and selection of protein in milk, albumin of egg (animal protein) or gram seed extract (plant protein) by performing the various tests given in the table below.

OBSERVATION AND RESULTS

S.No.	Test	Observation	Result
(i)	**Biuret Test**		
	(a) Take 2 ml of sample or test solution (milk/egg albumin/gram seed extract) in test tube + 1 ml Biuret reagent (containing 1 ml of 40% sodium hydroxide solution and 1-2 drops of 1% $CuSO_4$ solution). **(b)** Shake the tube thoroughly.	Pink, red or violet colour appears.	It shows the presence of proteins.
(ii)	**Xanthoproteic Test**		
	(a) Take 2 ml of sample or test solution(milk,eggalbumin/gram	White precipitate is formed.	It shows the presence of proteins.

	seed extract)in a test tube and add 2-3 drops of concentrated HNO_3 to it.		
	(b) Boil the solution over spirit lamp.	White precipitate changes to yellow precipitate	It shows the presence of proteins.
	(c) Cool the test tube and then add 2 ml of 20% NaOH (or ammonia solution) so that, it may become alkaline.	The colour of precipitate changes to orange.	It confirms the presence of proteins.
(iii)	**Millon's Test**		
	(a) Take 2 ml of test solution in a clean and dry test tube. **(b)** Add 2 ml of Millon's reagent.	Red-orange colour appears.	It shows the presence of proteins (mainly tyrosine).

Note: Millon's test is not specific for proteins as it detects other phenolic compounds as well, therefore, it must be confirmed by performing either Biuret test or Xanthoproteic test.

PRECAUTIONS

1. Use separate droppers for adding different reagents.
2. Care must be taken that excess of copper sulphate is not added in Biuret test otherwise, there will be blue colour instead of violet colour.
3. Proper care should be taken, while heating the samples in test tube. Keep the mouth of the test tube away from yourself and your friends.
4. Proper care should be taken, while using acids like HCl and,
5. Always run a suitable control by taking water in a test tube instead of protein sample.

VIVA VOCE

Q1. Name few sources of protein.

Ans. Pulses, egg white green beans and peas and dry fruits, etc.

Q2. Why is protein important component of our diet?

Ans. Proteins make building blocks of our body. They are important in muscle building.

Q3. Name one test, which shows presence of protein in food.

Ans. Xanthoproteic test.

Q4. Millon test is not so specific for proteins. Why?

Ans. It detects the presence of phenolic compounds along with proteins. So, it is not so specific.

Q5. How do one protein differ from other?

Ans. Protein are made of amino acids. So, their structure is differ in the presence or absence of different amino acids and their structural configurations

Q6. Why are only few drops of $CuSO_4$ solution added during Biuret test?

Ans. Only few drops of $CuSO_4$ solution should be added during Biuret test, because excess $CuSO_4$ will produce blue colour instead of violet colour.

Q7. Why does the skin turn yellow when it inadvertently comes in contact with HNO_3

Ans. The skin turns yellow when it inadvertently comes in contact with HNO_3 because the aromatic amino acid present in our skin protein forms nitro-complex (picric acid) in a reaction with nitric acid, which gives yellow colour to the area of contact. This reaction is called xanthoproteic reaction.

Q8. How much energy is released by degradation of 1 g of protein?

Ans. 5.6 kcal of energy is released by degradation of 1 g of protein.

Q9. What are the two major tests for the detection of protein in plant and animal samples?

Ans. Biuret test and Xanthoproteic test are the two major tests for the detection of protein in plant and animal samples.

Q10. What is the role of a peptide bond?

Ans. Peptide bond joins the amino and carboxyl groups of successive amino acids.

EXPERIMENT C

Aim

To test the presence of fats and its detection in suitable plant and animal materials.

THEORY

Fats can be defined as 'esters of fatty acids and glycerol'. These are insoluble in water, but soluble in organic and non-polar solvents like chloroform and benzene. Fats are a rich source of energy that yield 9 kcal of energy per gram of fat and are stored in body in the adipose tissues. Fats can be tested with Sudan III reagent, which gives specific red colour if fat is present in food. Besides this, three other tests are also recommended for fat test in food. These are Solubility test, Emulsion test and Translucent test.

MATERIAL REQUIRED

Test tubes, test tube stand, test tube holder, droppers, beakers, Sudan III, ethyl alcohol, water, chloroform, ether, benzene, oil, ghee or butter and groundnuts..

PROCEDURE AND OBSERVATIONS

1. Crush some groundnuts in water and filter their extract in a test tube.
2. Use this filtrate or other sources of fat like oil, ghee or butter, etc. for performing following different tests given in the observation table to check its presence.

S.No.	Test/Procedure	Observation	Result
(i)	**Sudan dye Test**		
	(a) Take 2 ml of oil or butter or groundnuts extract in a test tube + 1 ml of Sudan III. **(b)** Gently shake the tube. **(c)** The lipid layer will show change in colour, while layer of water will remain uncoloured.	Pink droplets appear in the solution.	It indicates and confirms the presence of fats.
(ii)	**Solubility test**		
	(a) Take 5 different test tubes and place small pieces of butter in each test tube. **(b)** Add 2 ml of water in test tube A, 2 ml of ether in test tube B, 2 ml of chloroform in test tube C, 2 mL of benzene in test tube D and 2 ml of ethyl alcohol in test tube E. **(c)** Shake thoroughly.	The piece of butter will dissolve in organic solvents like ether, chloroform, benzene and ethyl alcohol, but does not dissolve in water.	It shows the presence of fats as they are insoluble in water.

(iii)	**Emulsion Test**		
	(a) Take 2 ml of sample + 2 ml water in a test tube. **(b)** Gently shake the tube.	Pink droplets appear in the solution.	It indicates and confirms the presence of fats.
(iv)	**Translucent Test**		
	(a) Crush peanut or butter piece and rub it on a piece of white paper.	The paper becomes translucent.	It shows the presence of fats.

PRECAUTIONS

1. Small quantities of sample extracts and chemicals should be used to avoid wastage.
2. The test tubes should be washed thoroughly before and after use.
3. The organic solvents should be used under the guidance of teachers.
4. Proper care must be taken while using Sudan III because it may stain the clothes.
5. Use different droppers for different reagents.

VIVA VOCE

Q1. How will you differentiate between fats and oils?

Ans. Oil remains liquid at room temperature, but fats are solid.

Q2. Why fats get dissolved in organic solvents like acetone, benzene etc?

Ans. Fats have the property to get dissolved in organic solvents.

Q3. Name the reagent used for presence of fat in food material.

Ans. Sudan III.

Q4. What are the simplest forms of fat?

Ans. The simplest forms of fat are fatty acids and glycerol.

Q5. Which test for fats can be done without the use of chemicals?

Ans. When fat or oil is rubbed on white paper, it makes the paper translucent. This Translucent test for fats is done without using any chemical.

Q6. Will fat dissolved in an organic solvent give a positive result with Sudan III?

Ans. Sudan III will not give a positive result with organic solvent because the fat gets dissolved in it. But if fat is mixed with water. Sudan III will stain the fat molecules with red colour and will give positive result.

Q7. Which chemical gives the confirmatory test for fat?

Ans. Sudan III gives the specific red colour with fats, and is side also used as a confirmatory test for them.

Q8. Name two solvents that can dissolve fat in them.

Ans. Non-polar solvents like chloroform and benzene can dissolve fat in them.