KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

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B.SC., BIOCHEMISTRY

SUBJECT : METABOLISM OF CARBOHYDRATES AND LIPIDS PRACTICAL

SUBJECT CODE: 18BCU311

SEMESTER III 4H-2C

Instruction hours/week: L:0 T:0 P:4

Marks: Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

Course objectives

Students are able to understand about the quantitative analysis of

- carbohydrates
- enzymes
- lipid derivatives.

Course outcomes (CO's)

- Students are able to perform quantitative estimations of the macromolecules like carbohydrate, enzymes and lipids present in various samples.

1. Estimation of Blood glucose.
2. Sugar fermentation of microorganisms.
3. Assay of salivary amylase.
4. Isolation of lecithin, identification by TLC, and its estimation.
5. Isolation of cholesterol from egg yolk and its estimation.

SUGGESTED READING

1. Nelson, D.L. and Cox, M.M., (2013). Lehninger: Principles of Biochemistry 6th ed.,W.H. Freeman and Company (New York), ISBN:13:978-1-4641-0962-1 / ISBN:10:1- 4641-0962-1.

2. Devlin, T.M., (2011). Textbook of Biochemistry with Clinical Correlations 7th ed., John Wiley & Sons, Inc. (New Jersey), ISBN:978-0-470-28173-4.

3. Berg, J.M., Tymoczko, J.L. and Stryer L., (2012). Biochemistry 7th ed., W.H. Freeman and Company (New York), ISBN:10:1-4292-2936-5, ISBN:13:978-1-4292-2936-4.

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DEPARTMENT OF BIOCHEMISTRY

Subject : **Metabolism of Carbohydrates and lipids Practical**

Subject code: **19BCU311**

Details of the Experiments

Exp. No.	Name of the Experiment	Support Materials
1.	Estimation of Blood Glucose	W1
2.	Sugar fermentation of microorganisms	W2
3.	Assay of salivary amylase	W3
4.	Isolation of lecithin, identification by TLC, and its estimation	J1
5.	Isolation of cholesterol from egg yolk and its estimation.	J2

Web Reference:

W1: www. biochemden.com/anthrone method-carbohydrate determination

w2:www.amrila-cdu/1sub=3brch=f3&S1m=11398cnt=2

w3:biochemen.com/salivary-amylase-enzyme-activity-assay/

Journal References:

J1: Palacios LE & WangT. Extraction of egg yolk lecithin. Journal of the America oil chemists society. 2005, 82:565-569.

J2: Taber DF, Li R & Manson C. Isolation of cholesterol from an egg yolk. Journal of chemical Education, 2011, 88:1580-1581.

KARPAGAM ACADEMY OF HIGHER EDUCATION

COURSE NAME: METABOLISM OF CARBOHYDRATES AND LIPIDS PRACTICAL

COURSE CODE: 19BCU311

CLASS: II BSC BIOCHEMISTRY

BATCH-2018-2021

SYLLABUS

1. Estimation of blood glucose
2. Sugar fermentation of micro-organisms
3. Assay of salivary amylase
4. Isolation of lecithin, identification by TLC and its estimation
5. Isolation of cholesterol from egg yolk and its estimation

Experiment No: 1

Estimation of Blood Glucose – Anthrone Method

Aim

To estimate the amount of sugar present in a given unknown sample.

Principle

Hot acidic medium sugar is dehydrated to hydroxy methyl furfural. This compound found a green colour product with anthrone which was read at 630nm.

Reagents

1. Anthrone reagent

Dissolve 200mg anthrone on 100 ml of ice cold 95% H_2SO_4. It should be prepared freshly before use.

2. Standard Glucose Solution

500 mg of glucose was dissolved in 100 ml of distilled water.

3. Working Standard

10 ml of stock was diluted to 100 ml with distilled water.

Procedure

Into a series of test tubes pipette out 0.1 to 0.5 ml of working standard and labeled as S1 to S5. The given unknown solution was made up to 100 ml with distilled water mix well and from that 0.5 ml was taken in two test tubes and marked as U1 and U2 make up the volume of each test tube to 1 ml. Then, 1 ml of distilled water was serves as blank and add 4 ml of anthrone in all test tubes. Test tubes were treated in a water bath for 8 minutes. Cool rapidly and read the dark green colour solution at 630 nm.

Draw the standard graph by plotting concentration of standard on X-axis and absorption at Y-axis. From the graph, the amount of the unknown was calculated.

Table

S. no	Solution	Volume of solution (ml)	Concentration (µg)	Volume of water (ml)	Volume of Anthrone (ml)	Heated in a boiling water bath for 8 minutes	Optical Density at 630 nm
1	Blank	-	-	1.0	4.0		
2	Standard						
	S1	0.2	20	0.8			
	S2	0.4	40	0.6			
	S3	0.6	60	0.4			
	S4	0.8	80	0.2			
	S5	1.0	100	-			
3	Sample	0.1	-	0.9			
		0.1	-	0.9			
4	Unknown	1.0	-	-			
		1.0	-	0.5			

Experiment No: 2

Sugar fermentation of micro-organisms

Aim

To find the ability of micro-organisms to ferment the given carbohydrate.

Principle

A metabolic process performed by almost all types of bacteria is known as fermentation. This will result in the production of ATP, the ultimate energy source of the organism. This will happen either in the presence or absence of atmospheric oxygen. Bacteria utilize the nutrients in their environment to produce ATP for their biological processes such as growth and reproduction. The enzyme systems in bacteria allow them to oxidize environmental nutrient sources. Bacteria will use different energy sources in the medium depends on the specific enzymes of each bacteria. Many bacteria possess the enzymes system required for the oxidation and utilization of the simple sugar, glucose. Some bacteria have the ability to degrade complex carbohydrates like lactose, sucrose or even polysaccharides. Such bacterium should possess the enzymes that should cleave the glycosidic bonds between the sugar units and the resulting simple carbohydrate can be transported into the cell. Lactose is a disaccharide consisting of the glucose and galactose connected by glycosidic bond. The bacteria which produce the enzyme lactase will break this bond and thus release free glucose that can be easily utilized by the organism. The characteristics feature of the enzyme production in the bacteria enables them to use diverse carbohydrates and this will aid in the identification of unknown bacteria. Fermentation is best described by the degradation of glucose by Embden - Meyerhof pathway or Glycolytic pathway.

Materials Required

1. Phenol Red carbohydrate fermentation broth
2. Bacterial culture
3. Inoculation loop
4. Incubator (37°C)

Ingredients of the Fermentation Broth

1. Trypticase : 1 g
2. Carbohydrate : 0.5 g

3. Sodium Chloride: 0.5 g
4. Phenol red : 0.0189 mg

*Autoclave at 115^{o} C for 15 minutes

Inoculation of Bacterial Culture into the Phenol Red Carbohydrate Broth

1. Aseptically inoculate each labeled carbohydrate broth with bacterial culture
2. Keep the un-inoculated tubes as control tubes
3. Incubate the tubes at 18-24 hours at 37^{o}C
4. Observe the reaction

Precautions

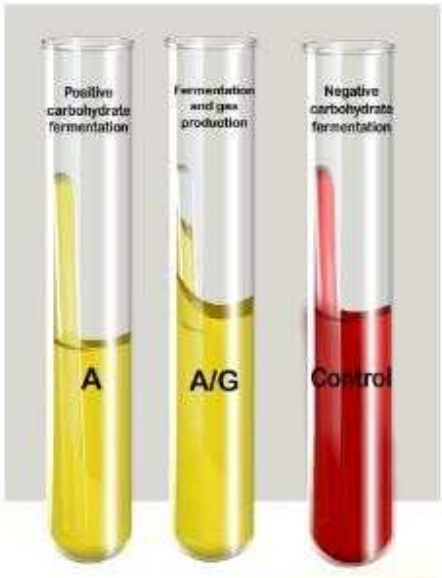
1. After inoculation into a particular sugar, sterilize the loop in order to avoid cross contamination of the tube with other sugars
2. Keep the un-inoculated sugar tubes as control tubes
3. Do not use the tubes with Durham tubes that are partially filled or with bubbles
4. Over incubation will help the bacteria to degrade proteins and will give false positive results.

Procedure

Preparation of Carbohydrate Fermentation Broth

1. Weigh and dissolve trypticase, sodium chloride, and phenol red in 100 ml distilled water and transfer into conical flasks
2. Add 0.5% to 1% of desired carbohydrate into all flasks
3. Insert inverted Durham tubes into all tubes, the Durham tubes should be fully filled with broth
4. Sterilize at 115°C for 15 minutes
5. **Important**: Do not overheat the Phenol red carbohydrate fermentation broth. The overheating will result in breaking down of the molecules and form compounds with a characteristic color and flavor. The process is known as caramelization of sugar (the browning of sugar)
6. Transfer the sugar into screw capped tubes or fermentation tubes and label properly

Expected Results



1. **Acid Production:** Changes the medium into yellow color - organism ferments the given carbohydrate and produce organic acids there by reducing the pH of the medium into acidic
2. **Acid and Gas production:** Changes the medium into yellow color - organism ferments the given carbohydrate and produce organic acids and gas. Gas production can be detected by the presence of small bubbles in the inverted Durham tubes
3. **Absence of Fermentation:** The broth retains red color. The organism cannot utilize the carbohydrate but the organism continues to grow in the medium by using other energy sources in the medium

Experiment No: 3

Assay of salivary amylase enzyme activity

Aim

To determine the activity of amylase enzyme in saliva

Principle

Amylase is the hydrolytic enzyme which breaks down many polysaccharides like starch, amylose, dextrins and yields a disaccharide i.e., Maltose.

$$(C_6H_{10}O_5)n + H2O \rightarrow n(C_{12}\ H_{22}\ O_{11})$$

Reagents

1. Substrate (starch) : Mix 1 gm of soluble starch in 200ml of 0.1M Phosphate buffer (pH 6.8) boil for 3 minutes and cool to room temperature and filter it necessary
2. Enzyme : Saliva is the best and easily available source of amylase. Collect some saliva in a beaker and dilute it to 1:20 dilution with distilled water
3. 1% sodium chloride : It is necessary for enzyme activity
4. DNS (Dinitro Salicylic acid) : Dissolve 1.6 gm of NaOH in 20 ml of distilled water. Take 1gm of 3,5 DNS in NaOH solution. In other beaker take 30 gm of sodium potassium tartrate. Dissolve in 50 ml of distilled water. Mix this DNS solution and finally make the volume up to 100 ml with distilled water.
5. Standard solution of Maltose: It is prepared by dissolving 200mg Maltose in 100ml of water (2mg/1ml).

Procedure

Take 0.5 ml of substrate and 0.2 ml of 1% NaCl in a test tube and pre-incubated at 37°C for 10 minutes then add 0.3 ml of diluted saliva and incubate for 15 minutes at 37°C. Stop the reaction by the addition of 1 ml of DNS reagent mix well and keep the test tubes in boiling water bath for 10 minutes. Cool and dilute with 10 ml of distilled water. Read the color developed at 520 nm. Simultaneously setup the blank as per the test by adding DNS prior to the addition of enzyme. Set up the standards of different test tubes and repeat the experiment as per the test and measure the color developed at 520 nm absorbance.

Preparation of Phosphate buffer

Dissolve 0.2M (2.7218 gms) of KH_2PO_4 in 100 ml of distilled water to this solution add M (2.8053 gms) KOH drop by drop till the pH is set to 6.8. Then make it to 200 ml with distilled water. So the final concentration is 0.1 M of 200 ml phosphate buffer.

Result

The amount of maltose in the given unknown sample is_____grams of maltose formed per 100ml of enzyme per one hour.

Table

S. no	Volume of standard (ml)	Volume of distilled water (ml)	Conc. of standard (ml)	Volume of DNS	Heated in a boiling water bath for 10 minutes	Volume of distilled water (ml)	Optical Density at 520 nm
1	Blank	1.0	0.0	1.0		10	
2	0.2	0.8	0.4	1.0		10	
3	0.4	0.6	0.8	1.0		10	
4	0.6	0.4	1.2	1.0		10	
5	0.8	0.2	1.6	1.0		10	
6	1.0	0.0	2.0	1.0		10	

Calculation

- 1.5 mg of maltose formed/0.3 ml/15 minutes
- 1.5 x 4 mg of maltose formed/0.3 ml of enzyme/1 hour
- 1.5 x 4 x 3.3 mg of maltose formed/1ml of enzyme/1 hour
- 1.5 x 4 x 3.3 x 100 mg of maltose formed/100ml of enzyme/ 1 hour

Experiment No: 4

Isolation of lecithin, identifying by TLC & its estimation

Aim

To isolate and quantify the amount of lecithin present in the given egg yolk.

Reagents

1. Ethanol ethyl ether mixture : 50 ml ethanol was mixed with 25 ml of ethyl ether
2. Acetone
3. Cadmium chloride : 0.5 g in 19 ml ethanol and 1 ml water (20 ml)
4. TLC plate

Procedure

In a separate beaker pour only egg white after cracking. Take egg yolk alone in 500 ml beaker. To the egg yolk added 50 ml of ethanol ethyl ether mixture and stirred vigorously for 5 minutes. Incubate it in room temperature for 15 minutes with occasional stirring. Then filter the precipitate with ethanol moistened filter paper in a separate dry beaker. Again the precipitate was washed with the remaining 25 ml of ethanol ethyl ether mixture and collected the liquid in a same container. Evaporate the filtrate by keeping the beaker in steam bath. The colloid substance obtained was transferred into the beaker containing 30 ml acetone slowly. Filter it immediately and the yellow precipitate obtained was dissolved in 20 ml of ethanol and 20 ml of cadmium chloride. Allow it to stand for 15 minutes and the cadmium salt of lecithin precipitate obtained was measured. Then it isolated lecithin was confirmed by running the TLC place.

Result

The amount of lecithin present in the given egg sample was found to be_____ mg.

Experiment No: 5

Isolation of cholesterol from egg yolk and its estimation

Aim

To isolate and quantify the amount of cholesterol present in the given egg yolk.

Principle

Egg yolk has a lipid to protein ratio of 2:1. The major lipid components are triacylglycerols, phospholipids and cholesterol. The major protein components are low-density lipoprotein, high density lipoprotein, and livetin (globular, water soluble), phosvitin (a phospho protein). Yolk from one large egg usually contains about 213 mg of cholesterol. In this experiment, qualitatively determine the lipids present in the yolk and quantify the amount of cholesterol in the yolk.

Extraction Procedure

1. Gently crack open the egg. DO NOT BUST THE YOLK.
2. Using the shell, separate the yolk from the white. Discard the white.
3. Add the yolk to a beaker (you can bust it now) and record its mass and volume. If you can, remove the white protein layer that the yolk is in. Scramble the egg.
4. Split the yolk into to 2 equal parts and perform the following steps

Acetone Extraction

1. Add an equal volume of acetone to each tube. This must be done in a glass centrifuge tubes.
2. Shake vigorously with vortexing for 2 minutes
3. Centrifuge
4. Collect the supernatant
5. Repeat 3 x more
6. Pool acetone fractions and allow acetone to evaporate in the hood.
7. The acetone extract should contain the cholesterol and yolk pigments. Save for your cholesterol assay.

Result

The amount of cholesterol present in the given egg was found to be_____mg.