Karmaveer Kakasaheb Wagh Education Society's
K. K. WAGH COLLEGE OF AGRICULTRURE,
Saraswatinagar Nagar, Panchavati, Nasik-422003

Theory Notes

Course Title : Biochemistry B. Sc. (Agri.)
Course No. : SSAC-354 Semesters: V
Credit : 3 (2+1)

DEPARTMENT OF SOIL SCIENCE AND AGRICULTURE CHEMISTRY

Teaching Schedule

Course Title : Biochemistry B. Sc. (Agri.)
Course No. : SSAC-354 Semesters: V
Credit : 3 (2+1)

Lect. No.	Topic	Weightage
1	History, scope and importance of biochemistry	4
2-3	Structure and organelles of plant cell and their role	3
4	Biomolecules - Definition, types, structure, properties and its applications	2
5-6	Carbohydrates - Definition, classification, structure, properties and functions.	5
7-8	Nucleotides and Nucleic acid - Definition, components and their structure	5
9-10	Amino acids, peptides and proteins - Definition, classification, structure and properties	6
11	Plant proteins and their quality, Essential amino acids and limiting amino acids	5
12-13	Lipids - Definition, classification, structure properties and their significance	4
14	Fatty acids- Definition, classification, structure and essential fatty acids	4
15-16	Biochemical energetics : Definition, free energy concept of chemical reaction, Components of electron transport chain, energy rich compounds	3
17	**MID TERM EXAMINATION**	
18-19	Enzymes- Definition, Classification, factor affecting enzyme activity.	5
20	Enzyme immobilization (inactivation) and its Industrial application in agro- industries	4
21	Vitamins and their coenzymes derivatives	3
22-23-24	Metabolic energy and it's generation metabolism - glycolysis, Citric acid cycle, Pentose phosphate pathways	4
25-26	Phosphorylation - Definition, cyclic and non-cyclic and substrate level phosporylatin, oxidative phosphorylations.	4
27	Fatty acid oxidation-β-oxidation	3
28-29	Biosynthesis- carbohydrates, lipids, proteins and nucleic acid	4
30	Metabolic regulation- integration of carbohydrate, lipid and protein metabolism	4
31-32-33	Secondary metabolites - glycosides, tannins, lignins, gums and mucilage-Definition, classification, properties and their physiolo -gical roles and application in food and pharmaceutical industries	4

34-35-36	Secondary metabolites -alkaloids, terpenoids - Definition, classification, properties and their physiological roles and application in food and pharmaceutical industries	4

PRACTICALS

Pract. No.	Name of practical
1-2-3	Qualitative tests for carbohydrates, proteins and lipids
4	Extraction of oil by Soxhlet's method
5	Estimation of protein by Lowry's method
6-7	Estimation of oil quality parameters (acid value, saponification value and iodine value)
8-9	Quantitative determination of reducing and total sugars by Benedict's method
10-11	Study of protein denaturation by heat, pH, precipitation of proteins with heavy metals
12	Determination of Ascorbic acid from fruit juice
13	Estimation of chlorophyll from plant sample
14	Separation of plant pigments by paper chromatography
15	Characterization of lipids by thin layer chromatography (TLC)
16	Determination of poly-phenols by Folin-Denis method
17	Study of amino acid models
18	Study of structural models of sugar- sucrose and starch

Reference Books:

1. Fundamentals of Biochemistry by J.L. Jain
2. Biotechnology by B.D., Singh
3. Principles of Biochemistry by Lehninger, Nelson & Cox
4. Outlines of Biochemistry by Conn & Stumpf
5. Textbook of biochemistry by A VSS, Ramarao
6. An Introduction to Practical Biochemistry by D.T. Plummer
7. Laboratory Manual in Biochemistry by Jairaman

LESSON PLAN -1

Topic : History and scope of plant biochemistry, important Biomolcculcs.

The term Biochemistry was first introduced by German scientist / chemist CARL NEUBERG in 1903.

Definition of Biochemistry

Biochemistry may be defined as a science concerned with chemical nature and chemical behaviour of the living matter.

Biochemistry may be treated as a discipline in which biological phenomenon are analyzed in terms of chemistry. Hence termed as biological chemistry or Chemical Biology.

History

In terms of history biochemistry is a young science.

Sr. No.	Period	Name of scientist	Contribution
1	1742-1786	Karl Wilhelm Scheele	Isolated citric acid, lactic acid, malic acid
2	1743-1794	Antoine Lavoisier	Father of biochemistry, developed the concept of oxidation of organic materials
3	1828	Wohler	Synthesized the first organic compound, urea from inorganic components
4	1854-1864	Louis Pasteur	Proved that fermentation is caused by microorganisms
5	1877	Kuhne	Proposed the term 'Enzyme'
6	1894	Emil Fischer	Demonstrated the specificity of enzymes and the lock and key relationship between enzyme and substrate
7	1897	Buckner	Discovered alcoholic fermentation in cell-free yeast extract
8	1902	Emil Fischer	Demonstrated that proteins are polypeptides
9	1903	Neuberg	First used the term 'biochemistry'

10	1913	Michaelis and Menten	Developed kinetic theory of enzyme action
11	1926	Sumner	First crystallized an enzyme, urease and proved it to be a protein
	1933	Embden Meyerhof and Parnas	Demonstrated crucial intermediates in the chemical pathway of glycolysis and fermentation
	1937	Krebs	Discovered citric acid cycle
	1940	Lipmann	Role of ATP in biological systems
	1950	Pauling and Corey	Proposed the α-helix structure for keratins
	1950-1953	Chargaff	Discovered the base composition of DNA
	1953	Sanger and Thompson	Determined the complete amino acid sequence of insulin
	1953	Watson and Crick	Proposed the double-helical model for DNA structure
	1958	Meselson and Stahl	Confirmed the Watson-Crick model of semi conservative replication of DNA
	1961	Jacob & Monod	Proposed the operon hypothesis and postulated the function of messenger RNA
	1999	Ingo potrykus	Golden rice- rich in β-carotene
	1838	Berzelius	Suggested the name proteins
	1822-1895	Louis Pasteur	Identified organisms responsible for fermentation.
	1852-1919	Emil Fischer	Studied structure of carbohydrates, Amino acids and fats.
	1906	F. G. Hopkins	Concept of deficiency diseases
	1912	Funk	Isolated and characterized the curative agent for scurvy (Vitamin – C), rickets (Vit. – D), Beriberi (Vit – B_1)
	1954	Watson and Crick	Helical model of nucleic acid
	1926	J.B. Sumner	First crystallized enzyme urease, Father of modern enzymology
	1935	Rose	Discovery of the first essential amino acid threonine.
	1929	Haworth	Formulation of sugars as pyranose form OR Furanose form

Scope of plant biochemistry

Biochemistry deals with study of

1. The nature of the chemical constituents of the living matter and the chemical substances produced by living things.
2. The functions and transformations of their chemical entities in biological systems.
3. The chemical and energetic changes associated with the transformation in the course of the activity of living matter.

Scope and importance of biochemistry in Agriculture

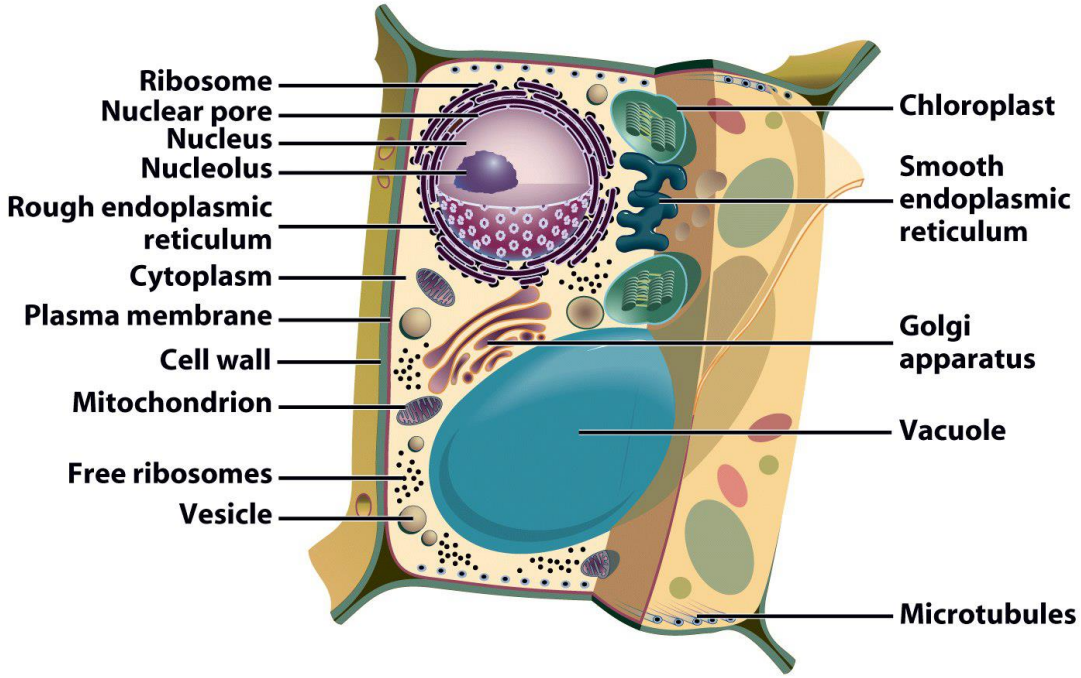
1) To evaluate nutritive value of cereals, pulses, poultry and cattle feeds.
2) Development and exploitation of better genotypes.
3) Removal and inactivation of toxic or anti nutritional factors present in food grains in general and grain legumes in particular by breeding and chemical treatments. e.g. BOAA in Lakh dal, Trypsin inhibitors of soybean, Aflatoxins of groundnut.
4) Food preservation and processing technology and post harvest physiology of fruit crops and vegetables and their nutritional quality.
5) Biochemistry of disease and pest resistance.
6) Biochemistry of drought resistance. Proline and hydroxyproline imparts drought resistance to Jowar.
7) Formulation of balanced diet.
8) Use of nonconventional sources of protein foods *viz*., single cell proteins, fish protein concentrates, mushrooms and leaf proteins.
9) Developments in the field of intermediately metabolism i.e. synthesis and degradation of constituents of living tissues.

Topic:3 Structures and functions of important cell Organelles, importance of water

Definition of cell

A cell may be defined as "Structural and functional unit of all living organisms". Two types of cells - 1) Eukaryotic cells 2) Prokaryotic cells.

Schematic diagram of a typical plant cell.



Important plant cell-organelles and their functions

1) Cell Wall - It Provides support, prevent cells from swelling and rupture or shrinkage, gives definite shape to cell.

2) Nucleus - Store of genetic information, which issue appropriate signal at proper time during different stages.

3) Mitochondria - Power house of energy, contain m-tRNA and DNA and protein synthesizing machinery, synthesis of ATP required for anabolism.

4) Chloroplast - The sites of photosynthetic phosphorylation. The stroma is the site of the carbon photosynthetic enzymes involved in CO_2 fixation, ribosomes, nucleic acid-synthesizing enzymes, and fatty acid synthesizing enzymes.

5) Ribosomes - Site of protein biosynthesis.

6) Golgi apparatus - Participate in the early stage of cell wall synthesis in higher plants. Site of secretions of proteins and polysaccharides and coupling of these two components to form glycoproteins. Intense phospholipid

biosynthesis observed in these organelles.

Importance of water:

i) Serves as a medium in which substances undergo fundamental changes.
ii) Provides hydrogen for the reduction of CO_2 in photosynthesis.
iii) Water is necessary reactant for the hydrolytic splitting of carbohydrates, fats and proteins.
iv) Water is solvent and dispersion medium for all protoplasmic constituents.
v) Acts as a transporting medium for all the cell nutrients.
vi) Absorption, secretion and excretion would not be possible without water.

Topic: 4
Biomolecules - Definition,types, structure, properties and its applications

Definition of Biomolecule: An organic compound normally present as an essential component of living organism.

IMPORTANT BIOMOLECULES

Characteristics of Biomolecules: -

1) Most of them are organic compounds.
2) They have specific shapes and dimensions
3) Functional group determines their chemical properties.
4) Many of them arc asymmetric
5) Macromolecules are large molecules and are constructed from small building block molecules.
6) Building block molecules have simple structure.
7) Biomolecules first gorse by chemical evolution.

Important Biomolecules of life

i) **Water -** Being the universal solvent and major constituents (60%) of any living body without which life is impossible. It acts as a media for the physiological and biochemical reactions in the body itself. Maintain the body in the required turgid condition.
2) **Carbohydrates -** It is very important for source of energy for any physical body function
3) **Proteins** - These are very important from body maintenance point of view, helps in tissue, cell formation.
4) **Lipids:** These are very important from energy source as well as human nutrition point of view.
5) **Nucleic acids -** Nucleic acids are very important as DNA carries the hereditary information and RNA helps in protein formation for the body.
6) **Enzymes -** Enzymes are simple or combined proteins acting as specific catalysts and activate the various biochemical and metabolic processes within the body.

Table - Fundamental Biological molecules (Biomolecules)

Sr.	Small molecules	Atomic constituents	Derived macro-molecules
1.	Amino acid	C, H, O, N (S)	Proteins
2	Sugars	C, H,O	Starch, glycogen
3.	Fatty acids	C, H, O	Fats, oils
4.	Purines and pyrimidine	C, H, O, N	Nucleic acids
5.	Nucleotide	C, H, O, N, P	Nucleic acids (DNA and RNA)

Topic : 5-6

Carbohydrates - Definition, functions, classifications, structure and Properties of Monosaccharide and Disaccharides.

Definition of carbohydrates :

Carbohydrates are defined as polyhydroxy aldehydes or polyhydroxy ketones and the substances which yield these derivatives on hydrolysis.

Functions of Carbohydrates

i) Supply energy
ii) Stored energy for future use
iii) Structural constituents
iv) Proteins sparing action
v) Necessary for oxidation of protein and fat
vi) Necessary for synthesis of non essential amino acids.
vii) Conserve water and electrolyte
viii) Beneficial effect on microflora.

Classification of carbohydrates

Carbohydrates are classified in to three major classes on the basis of complexity and behaviour on hydrolysis

1) Monosaccharides 2) Oligosaccharides 3) Polysaccharides

Monosaccharides :- Simple sugars and cannot be hydrolysed into smaller units. Depending upon no. of carbon in a unit, monosaccharides are subdivided into a dioses to decoses. More common subclasses of monosaccharides are: Based on the functional group, they are classified as aldoses and ketoses

1) depending on whether they have aldehyde or ketone as functional group

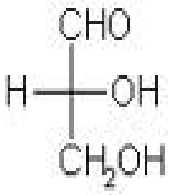
	Aldoses	**Ketoses**
Triose	Glyceraldehyde	Dihydroxy acetone
Tetrose	Erythrose	Erythrose
Pentose	Ribose, Xylose,Arabinose	Ribulose, Xylulose
Hexose	Glucose,Galactose, Mannose	Fructose
Heptose	-	Heptulose

Aldoses - Aldotrioses – e.g. Glycerose, **Aldotertroses** – e.g. Erythrose, **Aldopentoses** – e.g. Ribose **Aldohexoscs** – e.g. glucose, galatose **Aldoheptose** – glucoheptose.

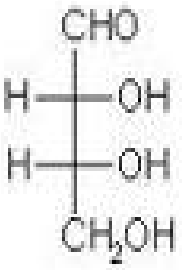
Ketoses - Ketotrioses – e.g Dihydroxyacetone **ketotetroses** – e.g erythrulose, **ketopentoses** - e.g Ribulose, **Ketohexoses**, e.g. Fructose, **Ketoheptose** e.g. Scdoheptulose.

Explain structure of triose tetrose, pentose and hexoses only

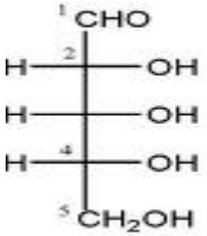
Aldoses



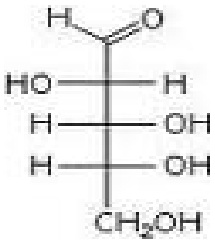
D-Glyceraldehyde



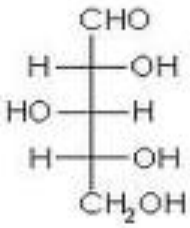
D- Erythrose



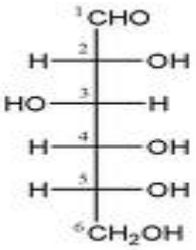
D-Ribose



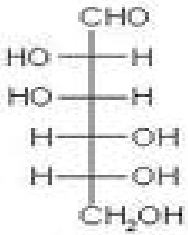
D-Xylose



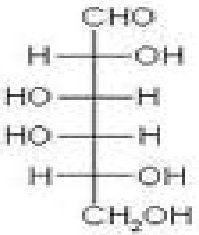
D-Arabinose



D-Glucose

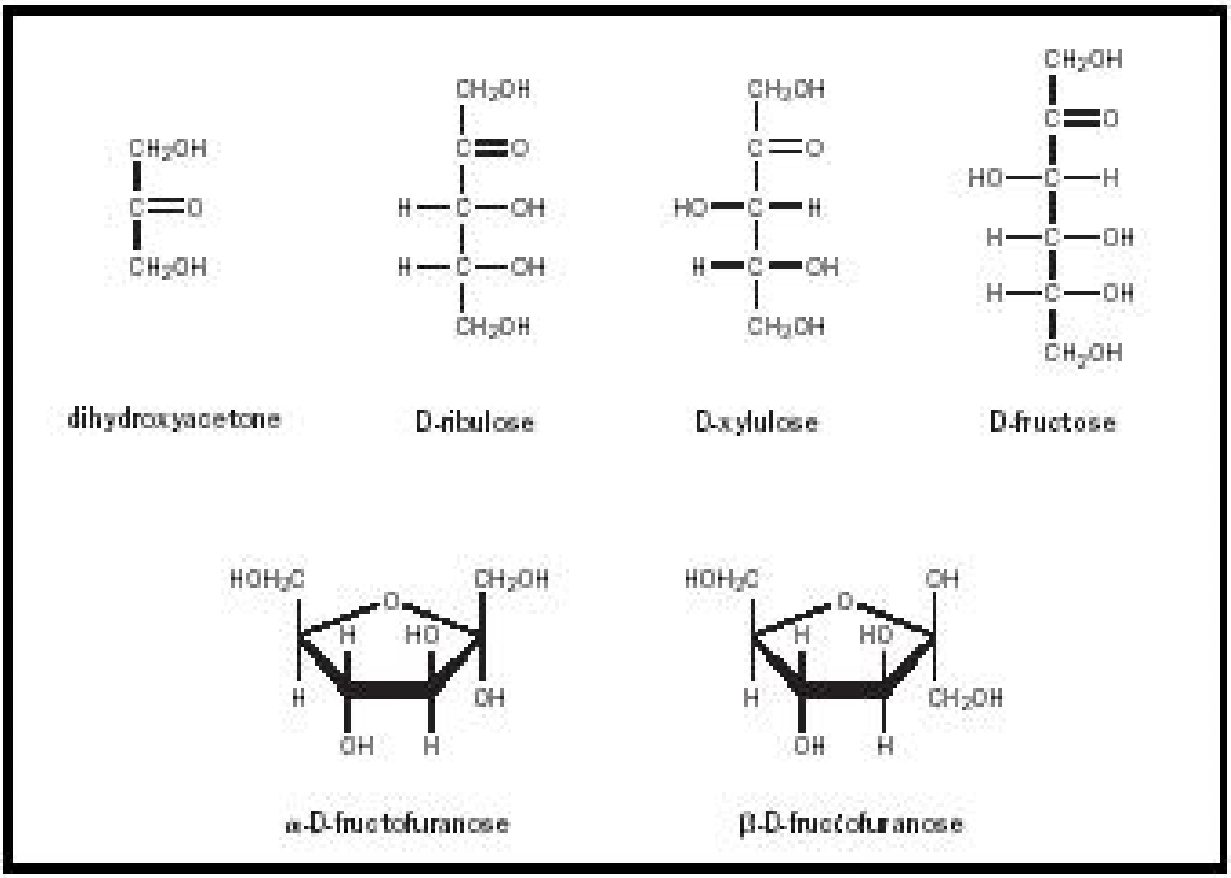


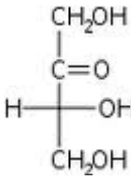
D-Mannose



D-Galactose

Ketoses



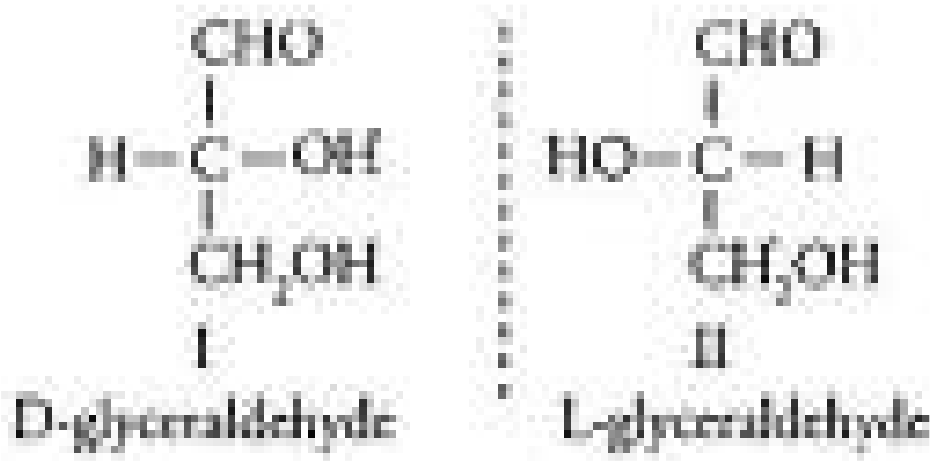


D- Erythrulose

Isomerism

a) *Stereoisomerism:* Most of the monosaccharides contain the same number of atoms and the same kinds of groups, yet they are definitely distinct substance. For example, the formula C6H12O6 represents 16 different simple sugars, all possessing the structure CH2OH. CHOH CHOH CHO. This is due to different arrangement of the constituent groups of the molecule in space. This phenomenon is called as stereoisomerism and these sugars are called as stereoisomers. For example; Glucose, mannose & galactose are stereoisomers. When there are several asymmetric carbon atoms in a chain molecule and the

end groups are not identical, the number of stereoisomer's possible is equal to 2n where n is the number of asymmetric carbon atoms. Thus there are 16 stereoisomers possible corresponding to the formula CH2OH. CHOH CHOH CHOH CHOH CHO, which contains four asymmetric carbon atoms (2n). Monosaccharide belong to D or L series depending on the position of OH group on the penultimate carbon atom. If OH is towards right side of the penultimate carbon atom it is called as D sugar and if OH is towards left side of the penultimate carbon atom it is called as L sugar. Glyceraldehyde, the simplest sugar is used as a reference compound for representing D & L forms of sugars. The structures of D and L glyceraldehydes are shown in the figure.

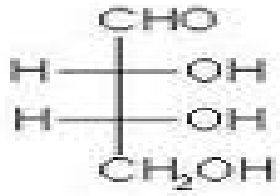


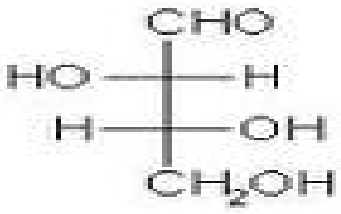
D & L forms of sugars which are non super imposable mirror images of each other are called *enantiomers.* Eg: D& L Threose molecules as shown in figure

D-Threose L-Threose

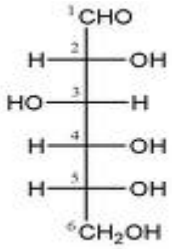
In nature, D-sugars are more widely distributed than L-sugars. The stereoisomers which are not mirror images of each other are called *diastereomers*

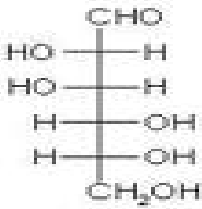
Eg: 1) D-Erythrose & D- Threose 2) D- Glucose, D- Mannose & D Galactose as shown in figure

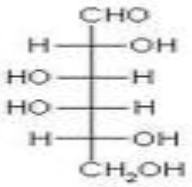




D-Erythrose D-Threose







D-Glucose D-Mannose D-Galactose

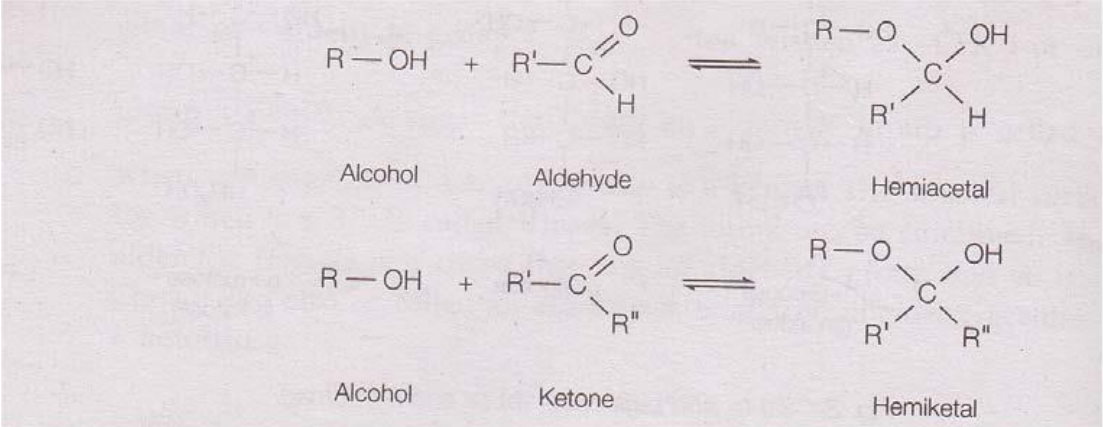
Among the diastereoisomers, those which differ in configuration at a single carbon atom are called *epimers*. Mannose is an epimer of glucose at 2nd carbon atom whereas galactose is an epimer of glucose at 4th carbon atom whereas galactose & glucose bear no epimeric relationship. The structural formulae of these sugars are shown above.

Structural isomerism: Some compounds have same molecular formula but different structural formulae. For example Glucose, galactose & mannose have same molecular formula but different structures and hence they are called structural isomers.

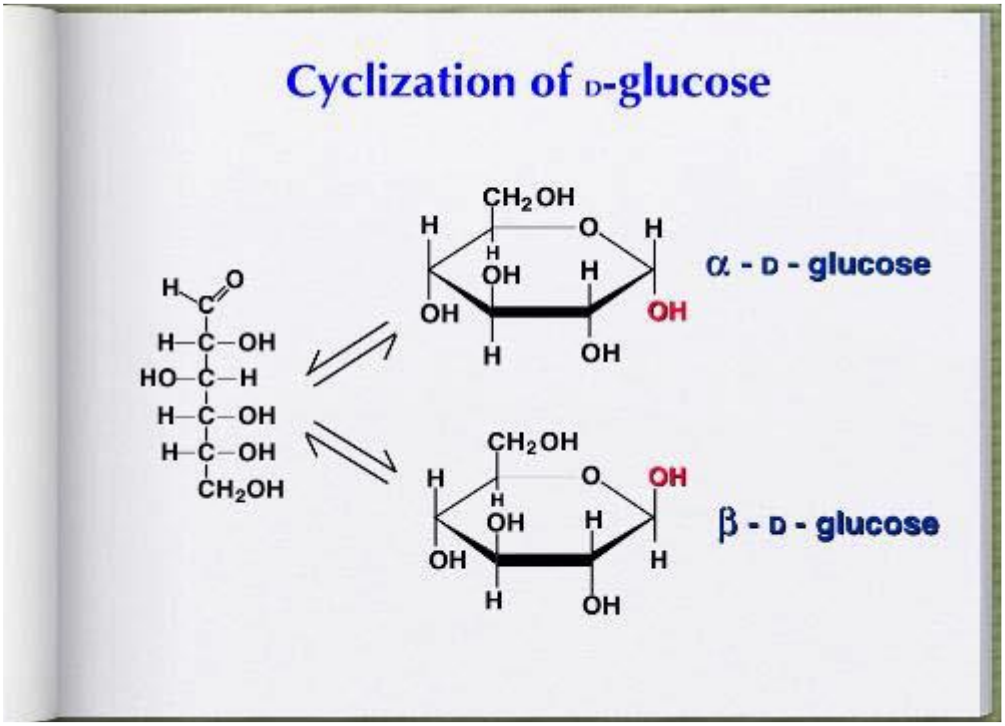
C)Functional isomerism: Glucose and fructose have same molecular formula but glucose is an aldose while fructose is a ketose. This kind of isomerism is called Functional isomerism.

d) Optical isomerism: Carbohydrates exhibit another kind of isomerism called optical isomerism. It is shown by the compounds having an asymmetric carbon. They have same molecular and structural formulae but differ in their behavior towards plane polarized light. An optical isomer rotating the plane of polarized light toward right is called dextrorotatory 'd' (+) while one rotating the plane of polarized light toward left is called levorotatory 'l' (-).

Ring Structures: The aldehyde or ketone group of a monosaccharide can react with a hydroxyl group to form a covalent bond. Formally, the reaction between an aldehyde and the hydroxyl group of a sugar (an alcohol) creates a hemiacetal structure whereas a ketone reacts with hydroxyl group of a sugar (alcohol) to form a hemiketal structure

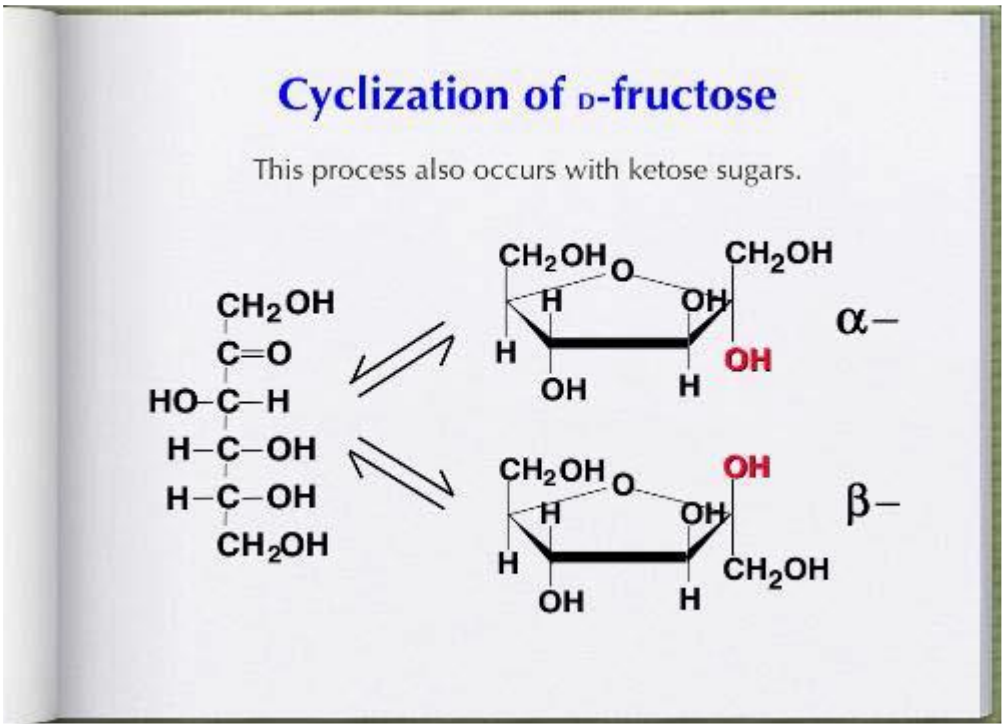


For tetroses and larger sugars, the reaction can take place within the same molecule so that the straight chain form of the sugar cyclizes. The following figure shows the cyclization of D-glucose to form a six-carbon ring.

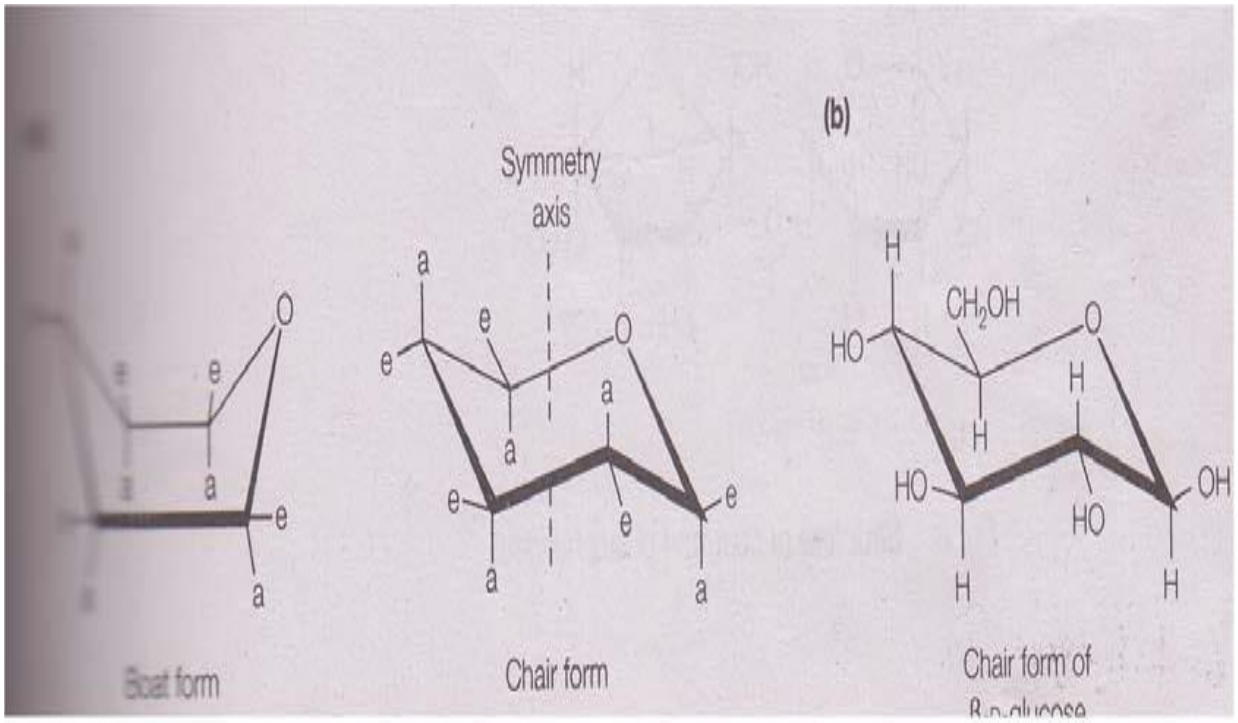


A new asymmetric center is formed during cyclization of an aldehyde at C- 1. Thus two isomers of glucose exists as α – D-Glucose in which OH group at C- 1 lies below the plane of the ring and β-D-Glucose in which the OH group at C-1 lies above the plane of ring. The C-1 carbon is called *anomeric* carbon atom and the alpha and beta forms are called anomers. In aqueous solution the alpha and beta forms are rapidly inter convertible via the open chain structure to give an equilibrium mixture and this is termed as *mutarotation*. Because of the structural similarity to the ring compound called pyran, the six membered ring structures of hexoses are called pyranoses.

Five membered sugars such as D-ribose and D-deoxyribose and six carbon ketose sugars such as D-fructose, form rings called furanoses as their Structures are similar to the furan ring. Again the furanoses can exists both in alpha and beta forms except here the nomenclature refers to the hydroxyl group attached to C-2 which is the anomeric carbon atom.



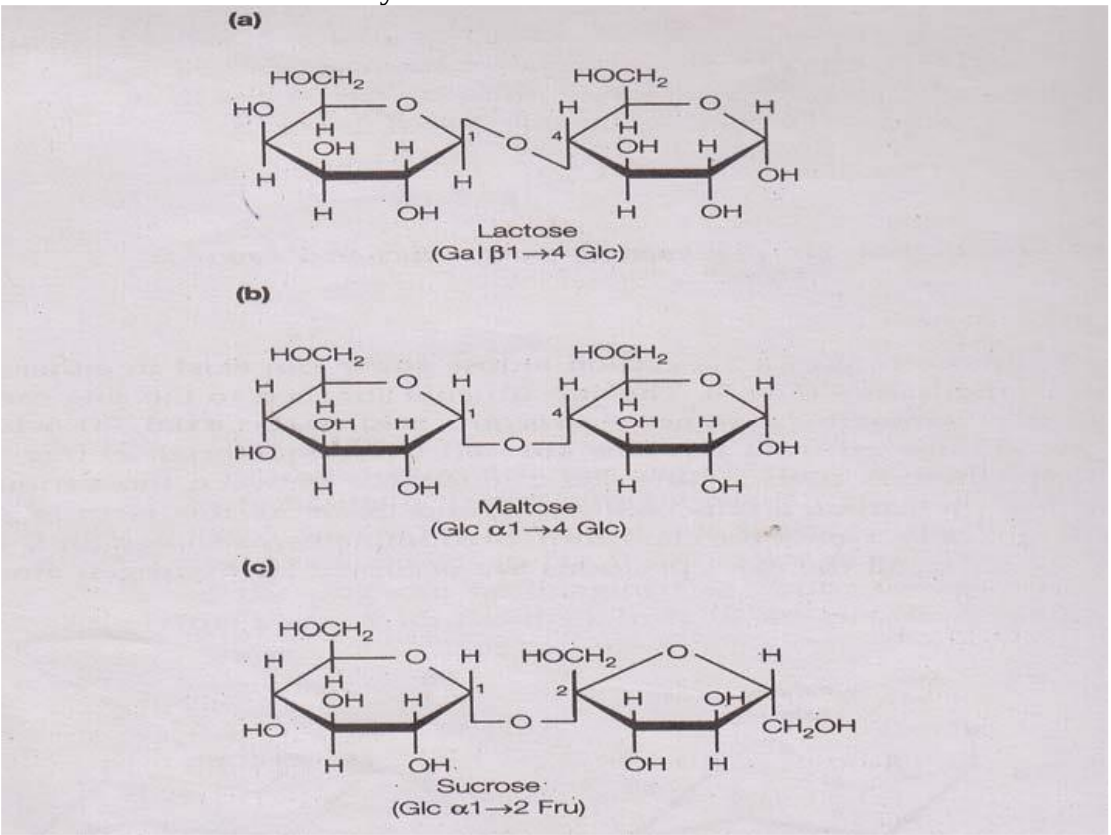
The pyranose ring of a six-carbon aldose sugar can exist in either a boat or a chair configuration. The substituents attached to the ring carbons that extend parallel to the symmetry axis are said to be axial (a) whilst those that extend outward from this axis are said to be equatorial (b) . In the boat form, there is considerable steric hindrance between the various groups attached to the carbon atoms of the ring and therefore this form is less favorable energetically. Hence the chair form predominates, as shown for β-D-glucose where all the axial positions are occupied by hydrogen atoms.



Oligosaccharides: The monosaccharide's condense with each other through glycosidic linkage to form oligosaccharides. The oligosaccharides are further classified depending upon the number of monosaccharide units present.
***Disaccharides*:** The hydroxyl group on the anomeric carbon atom of one Monosaccharide can react with the hydroxyl group of a second monosaccharide to form a disaccharide. The covalent bond formed is called a glycosidic bond.
Eg: ***a) Lactose*:** It is a disaccharide formed between the anorexic carbon C-1 of β-D-galactose and C-4 of α- D-glucose. Since the anomeric carbon of galactose molecule is involved in the bond and is in the beta-configuration, this is called β (1□4) bond which can be abbreviated as β1□4.
b) *Maltose*: It is a disaccharide formed between the C-1 and C-4 positions of two α- D glucose units. However, here the configuration of the anomeric carbon atom involved is the alpha form and hence the bond is called an α (1□4) bond or abbreviated as α1□4. For lactose and maltose, one of the anomeric carbons has been used to form the bond, leaving the second anomeric carbon free. Thus both lactose and maltose have a reducing end. Hence they are called as reducing disaccharides.

c) Sucrose: It is a disaccharide formed by glycosidic bond formation between the anomeric C-1 of α- D - glucose and the anomeric C-2 of β-D fructose so that sucrose lacks a free reducing group. Thus sucrose is a non reducing

disaccharide. It is formed by condensation of Glucose & fructose.



Trisaccharides: Three monosaccharide units condense with each other to form trisaccharides. Eg: Raffinose is formed by condensation of Galactose , Glucose & Fructose.

Polysaccharides: Many monosaccharide units condense to form polysaccharides through glysosidic linkage.

Polysaccharide classification:

They are classified depending on the function, nature of branching and repeating unit.

***1. Functional classification*:**

a. Structural polysaccharide: Polysaccharides belonging to this class help in maintaining the cell structure. Eg: Cellulose, chitin, Hemicellulose, pectin.

b. Storage polysaccharide: Polysaccharides belonging to this class help in storing carbohydrate material in the cell.

Eg: Starch, glycogen, inulin.

2. Nature of branching:

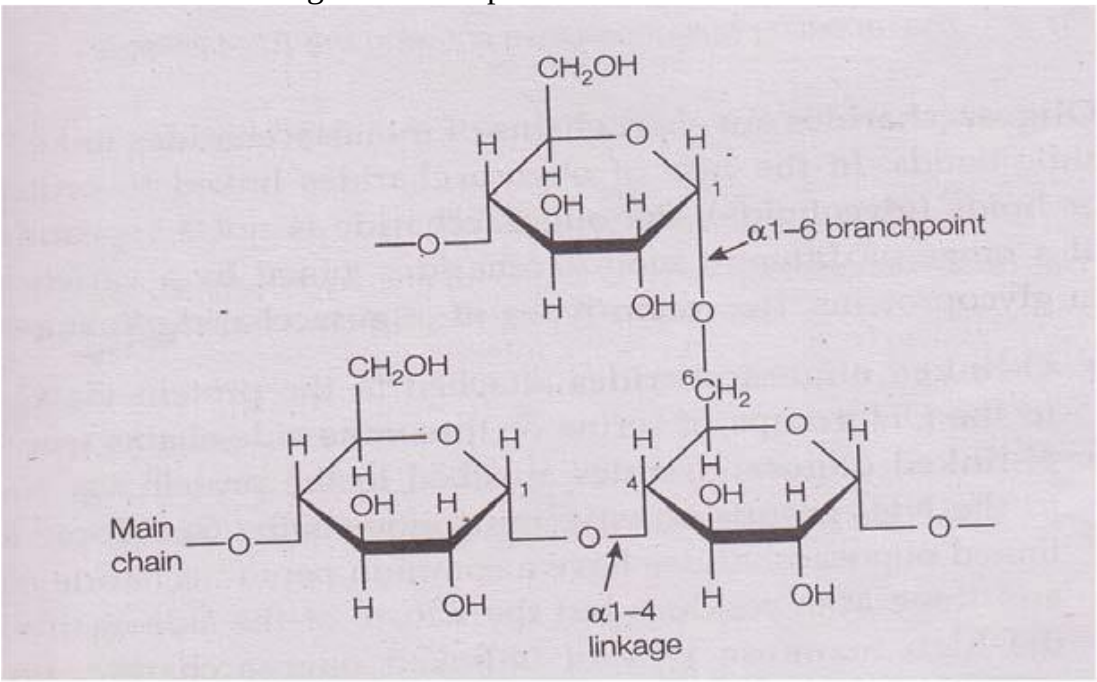
a. Linear: Polysaccharides belonging to this class have a linear glycosidic bonding only. Eg: Cellulose, chitin, amylose

b. Branched: Polysaccharides belonging to this class have a branched glycosidic bonding. Eg: Starch, amylopectin, glycogen.

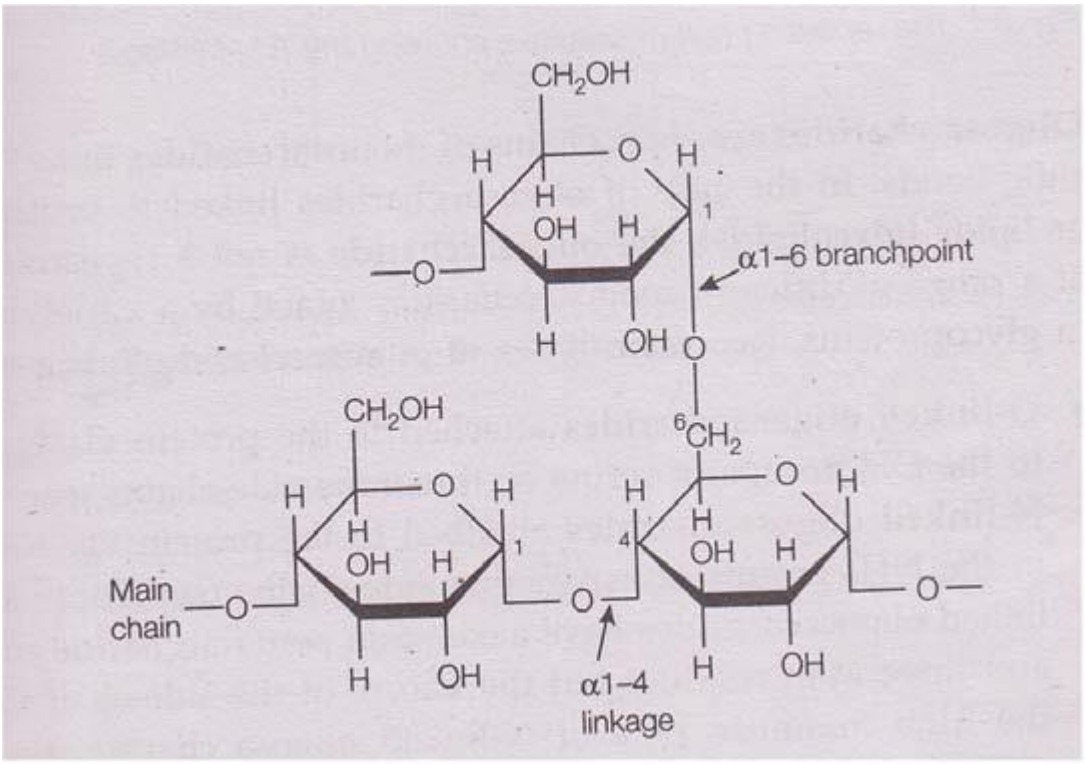
3. Repeating Unit:

a. Homopolysaccharide: Polysaccharides belonging to this class contain the same basic repeating monosaccharide unit. Eg:Starch, glycogen, chitin, inulin.
b. Heteropolysaccharides: Polysaccharides belonging to this class contain more than one basic repeating unit. Eg: Hemicellulose, pectin.
Polysaccharides are long chains of sugar units joined together.
Depending on the polysaccharide, the chains may be linear or branched. In plants, the storage form of glucose is the polysaccharide called as starch where as in animals excess glucose is stored as a large branched polysaccharide called glycogen. These polysaccharides serve as nutritional reserves and when required they are broken down and the monosaccharide products are metabolized to yield energy. In contrast, cellulose is present in cell walls and behaves as a structural polysaccharide.

***Starch*:** Starch exists in plants as insoluble starch granules in the cytoplasm. Each starch molecule contains a mixture of two polysaccharide forms, amylase and amylopectin. Amylose is unbranched polymer of glucose residues joined in α 1,4 linkages. Amylopectin is the branched form in which most of the glucose residues are joined in α 1,4 linkages but additional α 1,6 bonds occur at every 25-30 residues creating the branch points.

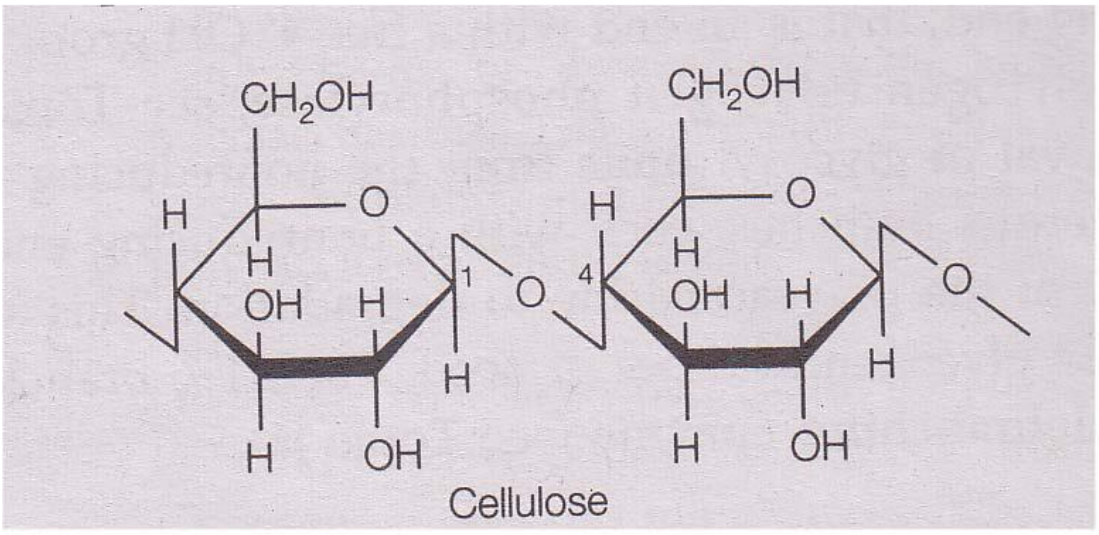


Glycogen: Glycogen molecule consists of glucose units which are linked in long chains by α1□4 bonds. For every 10 units or so, the chain is branched by the formation of α 1,6 glycosidic bond. The glycogen chain terminates in a non reducing end with a free 4'-OH group. Since the enzyme that degrades glycogen catalyzes the removal of glycosyl units from non reducing end of glycogen chain, the numerous branches, each with a non reducing end, greatly increase the accessibility of the polysachharide to degradation. The α1, 6 branches are removed by debranching enzyme.



Dextran is a glucose polymer where the glucose residues are mainly linked by the 1, 6 bonds. A few branches also occur which is formed by α1, 2, α1, 3 or α 1,4 bonds depending on the bacterial or yeast species that is the source of dextran.

Cellulose: Cellulose is an unbranched polysaccharide of glucose units linked by β 1, 4 bonds. The glucose residues in cellulose are arranged as straight fibrils. In plant cell walls, the cellulose fibrils are embedded in a matrix of other polysaccharides. In wood, the matrix also contains lignin, a complex polymer of phenolic residues. Mammals including humans, lack enzymes capable of digesting the β1,4 linkages of cellulose and so cannot digest plant cell walls.



Conjugated polysaccharides: Besides occurring in free state, the carbohydrates occur in nature in conjugation with other biomolecules like lipids and proteins to form glycolipids and glycoproteins.
Mucopolysaccharides are glycoproteins characterized by the presence of amino sugars like glucosamine, galactosamine.Eg: Hyaluronic acid & Heparin.

Industrial uses:

Monosaccharides, oligosaccharides and polysaccharides are used in number of industries as listed below:

Monosaccharides

1. Glucose and fructose are used as energy source
2. Liquid glucose is widely used in the confectionary, bakery, and jam preparation, canning and leather industries.
3. Glucose can be fermented to biofuel ethanol.
4. Liquid dextrose is used in fermentation industries, for the manufacture of dextrose monohydrates, fructose and sorbitol syrups.
5. Sorbitol syrup is widely used in tooth paste, pharmaceuticals, cosmetics and tobacco industries.
6. Fructose is used as sweetener in beverages, sport drinks and also used as a flavoring agent.
7. Fructose is used in cosmetic and pharmaceutical industry

Oligosaccharides

1. Sucrose is used in confectionery industry and in desserts.
2. Sucrose is used in preservation of foods.
3. Sucrose is used in cosmetic and pharmaceutical industry.
4. Maltose is used in baby food industry.

Polysaccharides

1. Food industry: Starch plays a leading role in determining the texture of many foods and texture is of vital concern to both the consumers and the manufacturers. Starch finds numerous uses in the baking industry for the production of cakes, cookies, in ice-cream preparations etc
2. Paper industry: In Paper industry, a large quantity of starch is consumed as a surface-sizing agent, as a binder, as a paper coating agent etc. Starch is used in the manufacture of various adhesives or glues for book-binding, wall paper adhesives, gummed paper, envelop adhesives, school glues and bottle labeling
3. Textile industry: In textile industry, starch is used in sizing to strengthen the warp yarn, in finishing and changing the appearance of fabric after it is bleached, dyed or printed. Starch is used as a component in finishing agent to glaze and polish sizing thread. Clothing starch or laundry starch is a liquid that is prepared by mixing a vegetable starch in water.
4. Pharmaceutical industry: Starch is used as an excipient, a binder in medications to aid the formation of tablets 5. Printing industry : In the printing industry, food grade starch is used in the manufacture of anti-set-off spray

powder used to separate printed sheets of paper to avoid wet ink being set off.
6. Plastic industry: Starch is used to produce various bioplastics,
Synthetic polymers that are biodegradable.

as shown in figureerties of Monosaccharides
1) Mutarotation 2) Glycoside formation 3) Reducing power
4) Reduction 5) Oxidation with mild and strong oxidizing agent
6) Methylation / Esterification 7) Dehydration
8) Form osazone with phenylhydrazine.

2.<u>Olignsaccharide:-</u> Definition - Oligosaccharides are polymers of monosaccharides containing two to ten residues accumulate in vacuole while polysaccharides in plastids, they are classified as

a) Disaccharides - yield two monosaccharides on hydrolysis.
i) Reducing disaccharides - e.g. Maltose (Glucose + glucose),
Lactose (galactose + glucose), Other examples are Isomaltose, cellobiose.
ii) Non reducing disaccharides - Sucrose (glucose + Fructose)

b) Trisaccharides - e.g. Raffinose - (Glucose + Fructose + galactose)
found in cotton seed and sugar beet. .

c) Tetrasaccharides - yield 4 monosaccharides on hydrolysis e.g. stachyose (glucose + Fructose + galactose + galactose) (only tetrasaccharide known to exist in plant).
Explain structure of sucrose, Lactose, Maltose only.

3. Polysaccharides :
<u>Definition of Polysaccharides</u>
Polysaccharides are polymeric anhydrides of monosaccharides. The long chain polymers are either straight chain or branched. They are also called glycanes.

Classification of Polysaccharides
1) On the basis of function 2) On the basis of composition
a) **Storage** e.g. Starch, glycogen a) Homopolysaccharides
b) **Structural** - e.g.Cellulose, Pectins. b) Heteropolysaccharides.

a) Homopolysaccharides - on hydrolysis gives single monosaccharide units
i) Pentosan - contains pentoses ($C_5 H_8 O_4$).
ii) Hexosans - Contains hexoses ($C_6 H_{10} O_5$) subdivided in to
A) Glucosans - Polymer or glucose e.g. starch, glycogen
B) Fructosans - Polymer or fructose e.g. inulin
C) Galactans - polymer of galactose e.g. Galactan
D) Mannans - Polymer of mannose e.g. Mananas.

b) Heteropolysaccharide - e.g. Hyaluronic acid, Chondroitin sulphates.

A) Gum - Consist of arabinose, rhamnose, galactose and glucoronic acid.
B) Agar - The sulphuric acid esters of galactans consists of galactose, galactouronic acid.
C) Pectins - Fundamental unit is pectic acid, consist of arabinosc,galactose, galactouronic acid.

Functions of Polysaccharides

1) They serve as structural components of the cells
2) They serve as stored form of energy
3) They serve as nutrient.

Structure and Properties of starch:- Consist of two components-Amyloses and Amylopectin. Amylose is a long chain polysaccharides containing α - D glucose molecules linked by 1- 4 glycosidic linkages, produce blue colour with iodine. Amylopectin is a branched chain polysaccharides consisting α-D-glucose molecules linked by 1-4-glycosidic linkage and branches by 1-6, linkage produce purplish colour with iodine and forms a gel with hot water.

Cellulose :- It is structural polysaccharide found in cell walls of plants, made up of long chains of α-D-Glucose molecules linked by 1-4 linkages, no branching, yield on hydrolysis crystalline D-glucose.

Pectin - Present in apple, lemon, form gel with sugar soln, contains, galacturonic acid, galactose and arabinose.

LESSON PLAN – 7 – 8

Topic : Nucleic acid - Definition, types, nucleoside, nucleotide. (N-base, sugar, phospbodiester bond, N glycosidic linkage), structure of DNA and functions

Definition of Nucleic acid

Nucleic acids are the polynucleotides having high molecular weight. The monomeric unit of which is nucleotide.

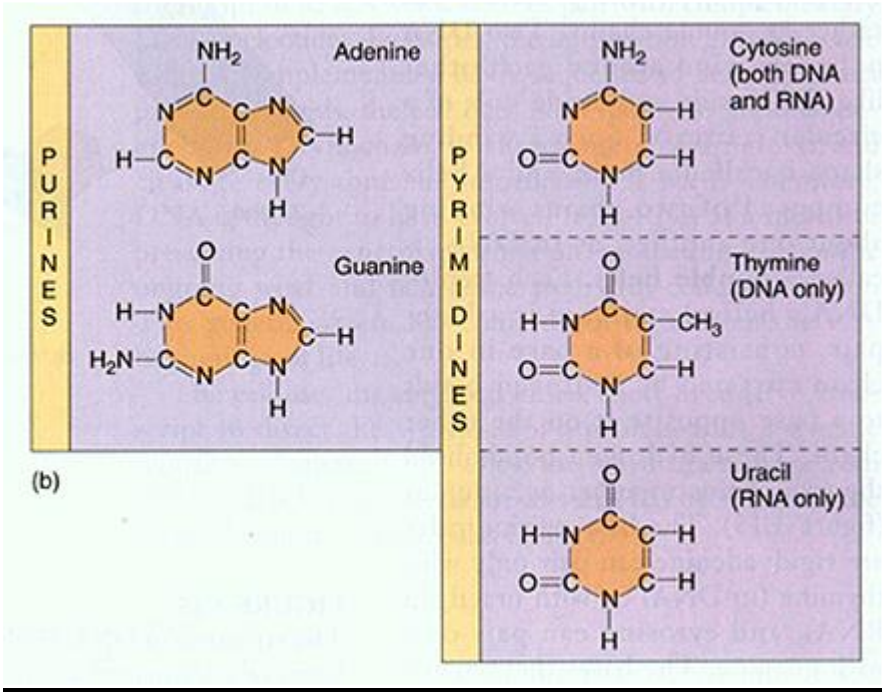
Nucleic acids were first discovered in 1868 by Friedrich Meischer. Nucleic acids are high molecular weight polymers which store and transfer genetic material from generation to generation. Knowledge of how genes are expressed and how they can be manipulated is becoming increasingly important for understanding nearly every aspect of biochemistry. These macromolecules are present in all living cells. Nucleic acids fall into two main classes according to the type of sugar they contain: the Deoxyribonucleic acids (DNA) & Ribonucleic acids (RNA).

Functions of nucleic acids:

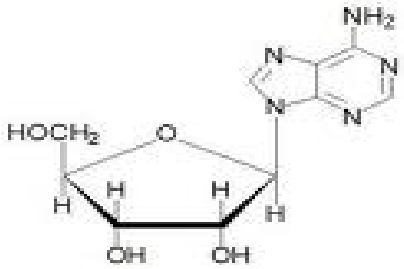
a) DNA stores and transmits genetic information
b) DNA expresses its encoded genetic information for the synthesis of RNA and protein for metabolic function.
c) DNA controls all cellular activities.
d) RNA is necessary for protein biosynthesis.

Nucleic acids are polymers of repeating units called nucleotides. Nucleotides are composed of Nitrogenous base, sugar and phosphoric acid. Nucleotides in nucleic acids are linked by 3’5’phospho diester linkages.

Nitrogen Bases: The five bases present in nucleic acids have carbon – nitrogen ring structures, hence they are called Nitrogen bases. There are two types of ring structures purines and pyrimidines. Adenine and Guanine are called purine nitrogen bases and Thymine, Cytosine and Uracil are called pyrimidine nitrogen bases. Pyrimidine nitrogen base present in both RNA & DNA is Cytosine, present only in RNA is Uracil and present only in DNA is Thymine.

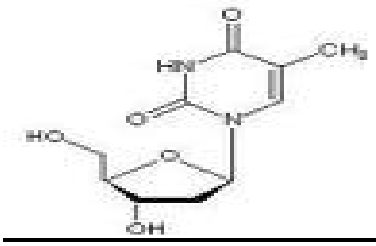


Nucleosides*:* The nitrogen bases are combined with ribose/deoxyribose to form nucleosides. In RNA, the nucleosides have ribose as sugar component and are called ribonucleosides, whereas they are called deoxyribonucleosides in DNA as the sugar in DNA is deoxy ribose. Nucleoside is formed by forming a bond between C1 of β sugar and N1 of the pyrimidine base or N9 of the purine base. This linkage is called as β - N glycosidic linkage

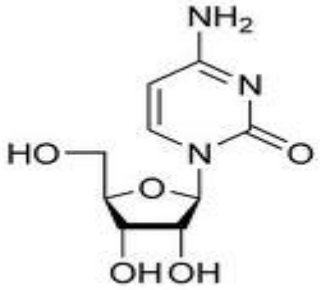


Adenosine

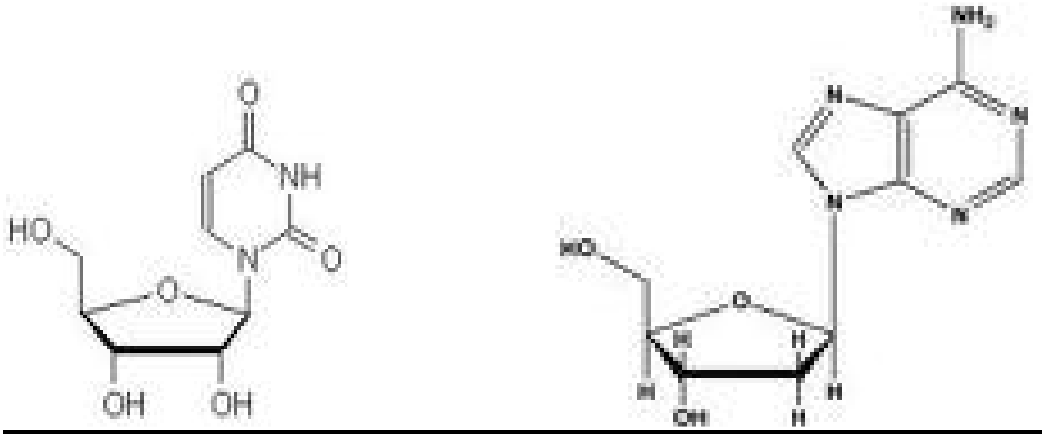
Guanosine



Thymidine

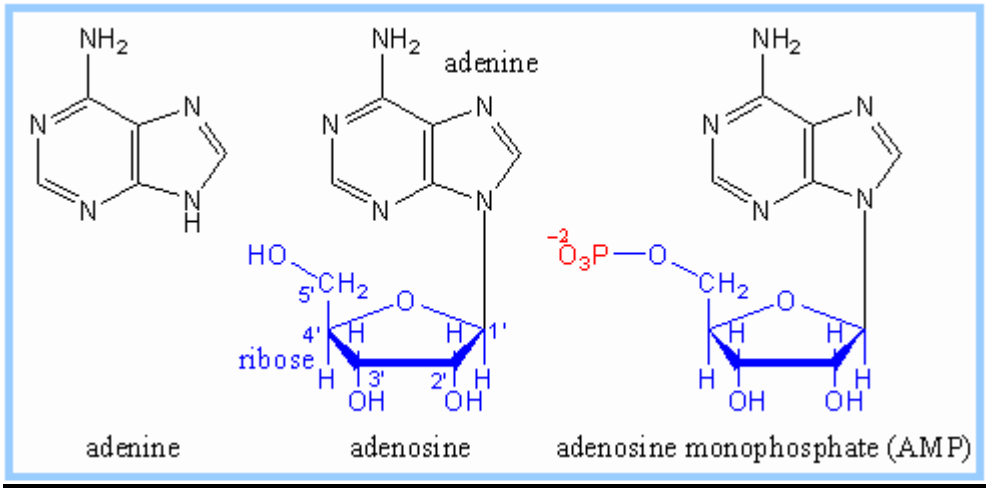


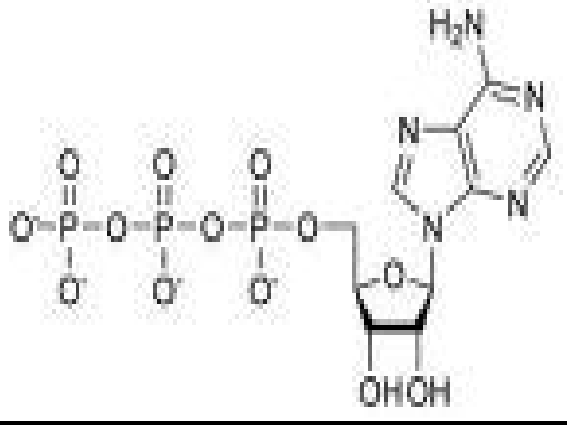
Cystine



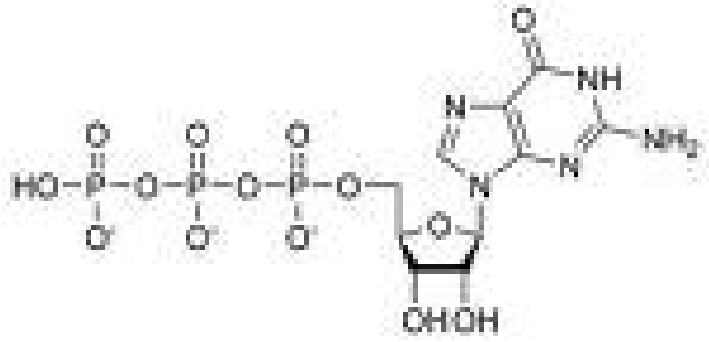
Uridine Deoxyadenosine

Nucleotide*:* A nucleotide is a phosphate ester of nucleoside. It consists of a phosphate group joined to a nucleoside at hydroxyl group attached to the C5 group of the sugar that is 5'-nucleotide. In DNA, the nucleotides have deoxyribose as the sugar and hence are called deoxyribonucleotides. In RNA, the nucleotides have ribose as sugar moiety and hence are called ribonucleotides. Deoxyribonucleotides & ribonucleotides can have a single phosphate group, two phosphate groups or three phosphate groups.

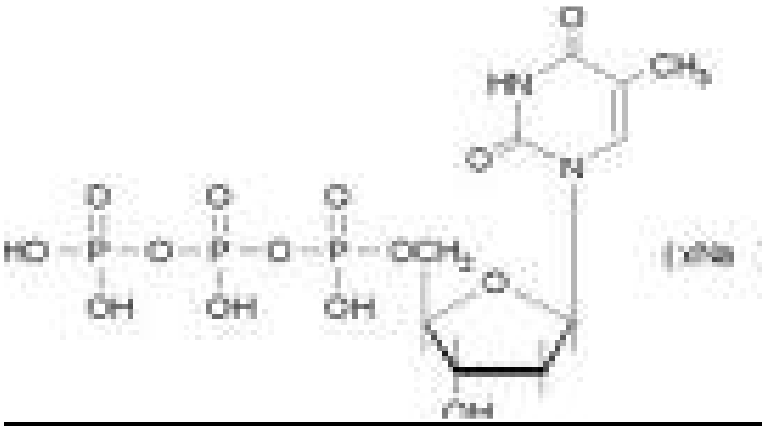




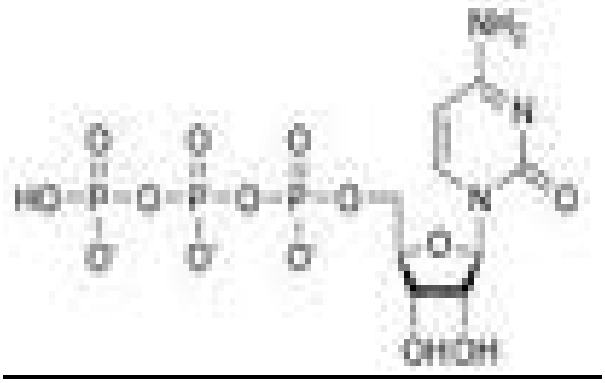
Adenosine triphosphate



Guanosine triphosphate



Thymidine triphosphate



Cytidine triphosphate

Types of nucleic acids

1) Ribonucleic Acid (RNA)
2) Deoxyribonucleic acid (DNA)

1) RNA - may be found in nucleus but mainly occurs in cytoplasm carry out protein synthesis work.
2) DNA :- Occurs in nucleus as well as cell organells like chloroplast and mitochondria.

Types of RNA

1) Transfer RNA (t - RNA)
2) Messenger RNA (m - RNA)
3) Ribosomal RNA (r - RNA)

Structure of Nucleic Acids

Nucleic acid components:
Sugar - ribose or dexyribose
Base + sugar = Nucleoside - N - glycoside bond.
Nucleoside + phosphoric acid = Nucleotide - Ester bond.
Nucleic acids- condensation polymer of nucleotide (Nucleotide-nucleotide)-phosphodiester bond.

Hydrolytic Products of RNA and DNA

Sr. No.	Components	RNA	DNA
1.	Pentose sugar	D-Ribose	D-2-deoxyribose
2	Acid	Phosphoric acid	Phosphoric acid
3.	Nitrogen bases a) Purines	Adenine Guanine	Adenine Guanine
	b) Pyrimidine	Cytosine Uracil	Cytosine Thymine

Watson -Crick double helical structure of DNA and forces responsible for stability of helix.

Functions of Nucleic Acids

1) Transmission of hereditary Characters (DNA)
2) Synthesis of proteins (RNA)

DNA - Store house of genetic information control protein synthesis in cell. Direct synthesis of RNA.
RNA - Direct synthesis of specific proteins.
m-RNA - To take genetic massage from RNA
t- RNA - Transfer the activated amino acids to the site of protein synthesis.
r- RNA - Function not clearly understood. Mostly present in ribosomes and responsible for stability of m-RNA.

Properties of Nucleic Acid

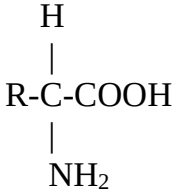
1) Optical property – Absorbance in UV at 260 nm
2) Melting temperature – T_m analysis

LESSON PLAN – 9

Topic : Amino acid - Classification, properties, Essential amino acids, peptide linkage

Definition of Amino acid

Amino acids are organic acids which contain both basic (amino - NH_2) and acidic (carboxyl COOH) groups and have general formula



Classification of Amino Acid

Based on composition they are classified as :

1) Aliphatic mono amino monocarboxylic acids.
   e.g. glycine. alanine. valine. leucine, isolucine
2) Aromatic amino acids - *e.g.* phenylalanine, tyrosine and tryptophan
3) Hydroxy amino acids *e.g.* serine. threonine
4) Acidic or dicarboxylic acids. e.g. Aspartic acid and glutamic acids
5) Basic amino acids - *e.g.* Lysine, arginine and histidine.
6) Sulphur containing amino acids e.*g.* methionine, cysteine
7) Secondary amino acids e.*g.* proline and hydroxyproline
8) Non protein amino acid e.g. Aminobutyrate, homoserine and cystathionine in plants.

Properties of Amino acids - Colourless, crystalline, tufts of slender needles (Tyrosine) to hexagonal plates (cystine). Taste varies from sugar sweet (glycine, alanine) through tasteless. (Tyrosine) to bitter (arginine)
All amino acids except glycine contain at least one asymmetric carbon atom ornithine needles (tyrosine) to hexagon plates (cysteine)

1) Solubility 2) Amphoteric nature 3) Esterification
4) Ninhydrin reaction 5) Reaction of alpha amino acid with HCl
6) Reaction of carboxylic group with NaOH 7) Zwitter ion
8) Electrical property

Amino group of one amino acid combine with carboxyl group of second amino acid linkage called peptide. Explain the term dipeptide, Tripeptide, tetrapeptide, polypeptide, Structure of Dipeptide.

Buret Reaction

Peptide containing two or more peptide bonds will react with Cu^{2+} in an alkaline solution to form violet blue colour.

Essential Amino Acids

Most of the naturally occurring amino acids arc indispcnsible. Naturally occurring ten amino acids can not be synthesized in animal body for which external supplementation is needed Ex. Tryptophan, Histidine, Arginine, Leucine, Isoleucine, Lysine, Valine, methionine, phenylalanine, Threonine.

Functions - i) Formation of proteins ii) Maintenance of tissues iii) Formation of enzymes, hormones and antibodies.

LESSON PLAN – 10

Topic - Proteins - definition, classification, properties' and functions.

Definition - Polymeric compounds the monomeric units. of which is amino acid.

Classification of Protein (with examples)

A) Based on composition B) Based on structure

A) Based on composition.

i) Simple proteins ii) Conjugated proteins iii) Derived proteins

i) Simple proteins - Classified according to solubility
   a) Albumins b) Globulins c) Glutelins
   d) Histones e) Protamine f) prolamines
   g) Scleroproteins

ii) Coniugated proteins :- Contain amino acid + prosthetic group.
   a) Glycoproteins b) Chromoproteins c) Lipoproteins
   d) Nucleoproteins e) Phosphoprotein

iii) Derived proteins :- Derivatives of proteins due to action of heat, enzymes, or chemical reagents.
   a) Primary derived b) secondary derived

B) Based on structure

i) Fibrous ii) Globular

Function :

1) Storage 2) Transport 3) Structural material
4) Metabolic growth regulator 5) Control of physiological functions
6) Catalytic activity 7) Hormonal 8) Toxicity by foreign proteins.

Properties of proteins :

i) Optical property. ii) Colloidal iii) Solubility
iv) Amphoteric nature v) Denaturation of proteins etc.

LESSON PLAN – 11

Topic : Plant proteins and their quality, Essential amino acids and limiting amino acids

(1) Between 10 and 30 % of the protein in a forage is converted into human food by ruminants, whereas 40 to 60 % of the protein can be extracted. The approximate consequences of fractionating a forage crop rather than using it as fodder.

(2) Leaves are the main site of protein synthesis and there are losses during translocation to other parts of a plant.

(3) When LP is made, the crop is harvested when less mature than when silage is made, and much less mature than when hay is made or a conventional crop is taken; the cost of harvesting is greater but an immature crop is not at risk for so long from diseases and pests.

(4) Crops that regrow several times after being cut young, or perennial crops, maintain cover on the ground; this enables fuller use to be made of sunlight and protects the ground from erosion.

(5) The fibrous residue contains the protein that was not extracted. Depending on the processing conditions, it can have two to five times as great a percentage of dry matter as the original crop and can therefore be dried to produce conserved ruminant feed economically.

Crops

Species and varieties selected for seed production or for a use other than LP extraction have been the source of most of the LP made in bulk. If varieties, possibly of species not at present used in agriculture, were investigated, yields would probably be greater than those so far attained.

Cowpea (Vigna unguiculata) gave 895 kg/ha in 80 days i.e., more than 4 tons if that rate could have been maintained for a year. In short term experiments yields as great as 17 kg of extracted protein per ha per day have been claimed.

Separation of extract from fibre

The yields given above were measured on 4 to 5 kg samples of leaf taken from within a crop, pulping them in the unit designed for **IBP** (Davys & Pirie, 1969), pressing a sample in the unit similarly designed (Davys, Pirie & Street, 1969) and measuring the amount of protein precipitable from the extract with trichloroacetic acid. In large-scale work it is usually advantageous to re-extract the-fibre, this can give half as much protein again as a single extraction, but it would be difficult in the laboratory to get quantitative and repeatable results from a double extraction. The manner in which increasing skill in

agronomy and processing have increased yields at Rothamsted.

Separation of protein from extract

Heat coagulation is generally accepted as the most satisfactory method for making a protein curd. Green, predominantly 'chloroplastic', protein coagulates at 50 to 60 ^{O}C ; if that is separated, colourless 'cytoplasmic' protein separates at 70 ^{O}C. No more protein coagulates on further heating, but heating to 100 ^{O}C is probably advantageous in other ways' it ensures a more nearly sterile product and it inactivates leaf enzymes more completely. When steam is injected into a stream of juice, heating takes place in 1 or 2 seconds; this produces a had, easily filtered cured, and there is less enzyme action before inactivation. Chlorophyllase-rich plants such as lucerne and wheat show the importance of this; the chlorophyll in LPs made by heating to 80°C was almost completely hydrolysed to chlorophyllide, whereas there was little hydrolysis during quick heating to 100°C (Arkcoll & Holden, 1973).

Bengal gram

The Bengal gram or chick pea (*Cicer arietinum*) has two principal cultivated types; the brown or yellow – brown Deshi type and the white seeded Kabuli type. Variation in their nutritive values are presented in table.

Table : *Composition of two types of Bengal gram*

	Crude protein (%)	Ether extracts (%)	Crude fibre (%)	Ash (%)	Carbo-hydrates (% by differ-ence)	Phos-phorus *(mg /* (%)100 g)	Calcium *(mg /* 100 g)	Iron *(mg/ 100* g)
Kabuli type	21.64	5.78	5.49	2.67	64.42	*305.8*	167.4	8.36
Deshi type	20.91	4.56	10.06	2.69	61.78	308.8	231.1	6.90

Red gram

Red gram or pigeon-pea is the second most widely cultivated pulse in India. Based on morphological characters, two forms, namely *Cajanus cajon var. flavus,* commonly known as tur and *Cajanus cajan* var. *bicolor,* known as arhar, have been described. The former type includes the commonly cultivated varieties, which are relatively dwarf and bear yellow flowers and plain pods; the latter type includes most of the perennial types, which are generally late-maturing, tall and bushy varieties.

Table : Methionine and sulphur content of varieties of red gram

Variety	Methionine (mg/g)	Sulphur (mg/g)
P.2780	3.00	1.30
P.3758	2.40	2.50
P.4768	2.20	2.90
P.4415	2.60	1.90
P.4657	2.30	1.72
R.24	2.05	1.92
S.32	2.05	1.32
S.34	2.03	1.72
Commercial varieties		
T-21	1.33	1.52
C-Il	1.80	1.70
N-84	1.55	1.50
T-15-15	1.60	1.50

Phaseolus group

Moong beans, urd beans and moth beans, are considered to be native to India, having been originated from *Phaseolus sublobatus* which grows wild in India.

***Moong bean or green gram* (*Phaseolus aureus*)**

The research work on the improvement of moong beans was started in India in 1925 with large collections of seed samples from different districts of the country and also from Burma. Pure line selection from the local materials resulted in some promising varieties, e.g., GG-127, GG-188, Krishna – 11, Khargone-l, Co. 1, Kopergaon, NP-23 and Jalgaon 781.

***Urd bean or black gram* (*Phaseolus mungo*)**

The earliest attempts to improve urd bean started in 1925, when 125 strains were isolated from the local bulks. Systematic improvement of urd was started in 1943. These efforts resulted in a number of promising varieties, both for dry areas, e.g., BG-379, B.R. 61, Mash-48, Mash 35-5, Khargone-3, T-27, T-65 and Sindh Kheda 1-1, and also for wetlands, e.g., ADT-1.

***Moth bean* (*Phaseolus acontifolius*)**

A breeding programme on this crop was started in 1943 and 150 collections were made from the cultivated areas of the country. From single plant selections, two types, namely B-15 and B-18, were identified as good grain types and T-3 as a good fodder variety. Another variety No. 88, was identified as a better grain type, maturing in 120 days. These lines showed

some improvement in yield, by 10-15 %, but no varieties resistant to diseases have been identified. Disease resistance and quality aspects are being considered in future breeding programmes.

Dolichos beans

Two major species of *Dolichos,* are commonly cultivated in India. One is *Dolichos lablab,* commonly known as walve or avare and the other *Dolichos biflorus,* known as horse gram or kulthi.

***WaIve or avare* (Dolichos lablab)**

Research work on improvement of avare has been carried out with the object of developing drought resistant, high yielding types with good quality pods. Some of the varieties, e.g., Co. 1, Co. 5 and Co. 6, have shown wide adaptability and are being popularized in rotation with late paddy in areas where winters are mild.

***Horse gram or kulthi* (Dolichos biflorus)**

Very little work has been done on the improvement of horse gram; however, as a result of single plant selections from the local bulks, a number of varieties recording 15-20 % more yield than local bulks have been developed. Some of the varieties, e.g., BGM 1-1, No. 35, D.B. 7 have been found promising. Variety BGM-1 exhibited a high degree of virus resistance.

Cowpea

A breeding programme for improvement of this crop *(Vigna sinensis)* has been in progress since 1940. A number of grain, fodder and vegetable varieties have been identified from time to time largely from collections made within the country or from abroad. of the grain types, N.P. 2, N.P. 7, C-32, T-I, K-11, K-14; of the fodder varieties.

Pea or matar

There are two main types of cultivated pea *(Pisum sativum),* namely the large, smooth or wrinkled-seeded garden pea and the small, round or dimpled-seeded field peas. While the former type is used as a table variety, the latter is used as pulse, whole or split.

Garden pea

A breeding programme on the garden pea was initiated at the Indian Agricultural Research Institute in the thirties. Through single plant selection, the medium-tall, wrinkled-seeded variety NP-29 was developed which is still popular in the country for its quality. During the same period, green-seeded Hara Bauna and white round-seeded Lucknow Poniya were popularised for general cultivation in northern India. In central India, where the winters are comparatively short, the variety Khapar Kheda became more popular. In the warm-temperate zone around the Himalayas, a smooth, white-seeded variety was popularised under the name Kala Nagini or Kanawari. In recent years, a

few more varieties, e.g., Early Badger, Boneville and Perfection with very attractive pod size have been introduced for general cultivation.

Lentil

Varietal improvement programmes for this species *(Lens esculentus)*, were initiated in India in 1924 by collecting mixed samples bought in bazaars all over the country. Single plant selections were picked up from the bulk population and sixty-six types were isolated. Some of these varieties, e.g., N.P. 11, N.P. 47 (IARI), T-36, T-8 (UP.), L-9-12 (Punjab) and B.R. 25 (Bihar).

Khesari or teora

The consumption of this pulse *(Lathyrus sativus)*, in large quantities leads to lathyrism because of the presence of *β*-N-oxalyl amino alanine *(βOAA)*. It is a very hardy crop and comes up well even under water-logging and extreme drought conditions. Therefore in areas which are completely dependent on the monsoon, farmers insist on growing it.

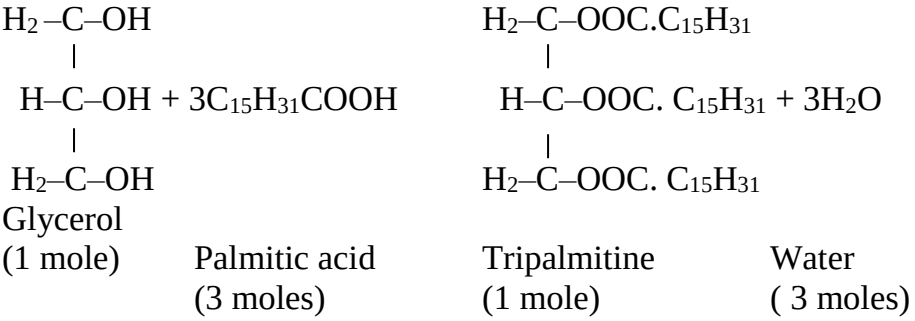
Topics : Lipids, Definition, classification, structure and chemical properties.

The term lipid was first used by the Gcnnan biochemist Bloor in 1943.

Definition of lipid.

Chemically lipids are defined as esters of glyccrol and fatty acids or as the triglycerides of fatty acids,

General formula of lipid = Glycerol + Fatty acid = Triglycerides



Glycerol (1 mole)	Palmitic acid (3 moles)	Tripalmitine (1 mole)	Water (3 moles)

Classification of Lipid

A) On the basic of reaction with NaOH / KOH

i) Saponifiable ii) Non saponifiable

B) On the basis to products of hydrolysis.

1) Simple Lipids : On hydrolysis gives fatty acids and alcohol (trihydric or monohydric)

Oils	: Unsaturated fatty acid + glycerol.
Fats	: Saturated fatty acids + glycerol,
Waxes	: Fatty acids + mono or dihydric alcohol.
Simple glyceride	: Contains same fatty acids.
Mixed glyceride	: Contains different fatty acids.

2) Compound lipids: (Complex lipids)

On hydrolysis gives phosphoric acid, Vararious sugars, sphingosine, ethanolamine and serine in addition to fatty acids and glycerol.

a) *Phospholipid* :- Fatty acids + glycerol + phosphoric acid + nitrogenous base.

e.g. Lecithin : Fatty acids + glycerol + phosphoric acid + choline

Cephalin : Fatty acids + glycerol + phosphoric acid + ethanolamine.

b) *Glycolipids* :- Glycerol + fatty acid + Carbohydrates (on hydrolysis)
They are sub classified as galactosyl diglyceride, cerebrosides and sulpholipids.

c) *Sphingophosphoipld*s : Fatty acids + sphingosine + phosphoric acid + choline.

3) Derived lipids : Hydrolytic products of simple and compound lipids,
i) Alcohols : Glycerol and other sterol
ii) Fatty acids
iii) Terpenoids.

Chemical properties of lipids

i) Hydrolysis	ii) Hydrogenation	iii) Halogenation
iv) Saponification	v) Oxidation	vi) Rancidity (oxidative and hydrolytic).

LESSON PLAN – 14

Topic : Fatty acids - Definition, classification, functions, essential fatty acids.

Definition of Fatty Acids

Fatty acids may be defined as organic acid that occur in a natural triglyceride and is a monocarboxylic acid ranging from C_4 to C_{28} atoms in straight chains and will usually have either a saturated hydrocarbon chain or may contain from one to six double bonds.

Classification

1) Saturated fatty acid :- Contain no double bond, chain is saturated.
e.g. Butyric acid (4), Caproic acid (6) Caprylic acid (8) Capric acid (10), Lauric acid (12), Myristic acid (14), Palmitic acid, (16) Stearic acid (18), Arachidic acid (20) Behenic acid (22) Lignoceric acid (24) , Cerotic acid (26) Montanic acid (28) **[Figures in the bracket is number of carbons].**

2) Unsaturated fatty acid :- Contain one or more double bond in chain and degree of unsaturation depends on the no. of double bond present in it.

Sub classified on the basis of degree of unsaturation.

a) Monoethenoid acids - contain one double bond e.g. oleic acid, palmitoleic acid
b) Diethenoid acids - contain two double bond e.g. Linoleic acid.
c) Triethenoid acid - contain three double bond e.g. Linolenic acid Eleostearic acid
d) Tetraethenoid acids - contain four double bond e.g. Arachidonic acid.

3) Branched chain fatty acids - Contain hydroxyl group in chain of fatty acid
4) *Cyclic fatty acid* – posses ring structure e.g. chaulmoogric acid and hydnocarpic acid

<u>Functions of fatty acids:</u>
1) Source of energy in stored forms
2) Solubilize vitamins like A, D, E, and K.
3) Building units of majority of lipids
4) Constituent of phospholipids

Essential fatty acids

Fatty acids which cannot be synthesized by the cell of the body must be obtained - from other source. Essential fatty acids arc linoleic, linolenic and arachidonic acid.

<u>LESON PLAN – 15 - 16</u>

Topic : Bio-chemical energetics. Definition, free energy concepts of chemical reaction, components of electron transport chain.

Definition of Bio-chemical energetics or Bioenergetics.

Study of the interconversion of forms of energy in biological system.

The concept of free energy.

Free energy (G) - The component of the total energy of a system that can do work at constant temperature and pressure.

Free energy change *(*ΔG*)* - The amount of free energy released (negative ΔG*)* or absorbed (positive ΔG*)* in a reaction at constant temperature and pressure.

The first law of thermodynamics states that energy cannot be created or destroyed but this law cannot be used to predict whether a reaction can occur spontaneously.

The second law of thermodynamics states that a process can occur spontaneously only if the sum of the entropies of the system and its surroundings increases.

Since the entropy changes of chemical reactions are not readily measurable, the entropy is not used as a criterion whether a biochemical process can occur spontaneously or not.

In 1878, Gibbs created the free energy function by combining the first and second laws of thermodynamics in the form of following equation.

$\Delta G = \Delta H - T\Delta S$

Exergonic reaction- spontaneous reaction, Δ G value is negative. Do not need energy supply from outside. Breakdown reaction.

Endergonic reaction - Non spontaneous, thermodynamically unfavourable reaction. Δ G is positive or more than zero. Reaction will occur only when energy supplied to the system. Synthetic reaction.

Energy rich compounds

Developed by Fritz Lipman, characterized by high energy bonds which have large free energy of hydrolysis. Energy rich compounds are symbolized by wriggle bonds.

1) Low energy bonds Δ G value is - 1 to -5 Kcal/mole.
2) High energy bonds Δ G value is -7 to -15 Kcal/mole.

Exhibit a large decrease free energy (Δ G) on hydrolysis e.g. creatine phosphate, arginine phosphate, phosphoenol pyruvate, Acetyl phosphate, ADP, ATP, Acyl. CoA. (With energy value)

Structural details of mitochondria and chloroplast.

Redox potentials of electron of carriers and calculation Δ G

Coupling of reactions - In the cell, the energy released or made available in an exergonic reaction is utilized to drive other endergonic reactions and thereby made to do works. The only way this can occur is by common reactants in a process known as the coupling of reactions.

Explain electron transfer system (ETS) with diagram

Components of electron transport chain.

1) Nicotinamide nucleotides 2) Flavoproteins
3) Nonheme iron proteins 4) Quinones
5) The cytochromes

LESSON PLAN – 18 - 19

Topic : Enzymes - Definition, classification (IUB system) Mechanism of action, nature and properties of enzymes, factors attesting enzyme activity.

Definition of Enzyme

Catalytically active protein of biological origin or organic catalyst produccd by living cells.

Substrate :- Substance upon which an enzyme acts.

Classification of Enzyme (IUB System, 1961)

6 major classes based on type of reaction

1) **Oxidoreducctase (Dehydrogenases)**
   Reaction catalysed - Biological oxidation – reduction - ex. Pyruvic oxireductase, Ascorbic oxidase, succinic dehydrogenase, cytochrome oxidase
2) **Transferases (Transaminase)** - Ex - Transketolase, transmethylase
   Rcaction catalysed - Transfer of groups.
3) **Hydrolases -** Ex- Lipases, urease, pepsin, anylase, cholinesterase.
   Reaction catalysed - Hydrolysis
4) **Lyases (Decarboxylases) -** Ex - carboxylases, aldolases, fumarase.
   Reaction catalysed - catalysed removal of groups
5) **Isomerases -**Ex -triose phosphate isomerase, - epimerase, alanine racemase.
   Reaction catalysed - Catalyse convcrsion to other isomeric form.
6) **Ligases** or synthetases Ex. Succinic thiokinase, Glutamine synthetase.
   Reaction catalysed - catalyse linking together of two compounds.

Mechanism of Enzyme Action

The enzyme combines with the substance on which it acts (substrate) to form an enzyme - substrate complex called Michaelis complex

$$E + S \longrightarrow E S$$

Enzyme is liberated and the substrate is broken into the products of the reaction

$$ES \longrightarrow E + P \text{ (products)}$$

Active site :- The region of an enzyme surface that binds the substrate molecule and catalytically transforms it.

Functional groups present at the active site.

Amino acids such as aspartic acid, glutamic acid, lysine, serine etc. the side chain groups - COOH, - NH_2, - CH_2OH etc. serve as catalytic groups at active site.

Fischer's lock and key model
Activation energy with and without enzyme

Chemical nature of enzyme
All enzymes are protein in nature without any exception. Most enzymes contain protein and non-protein groups.
Enzyme $\longrightarrow$ Protein + Non protein
Holoenzyme -- Apoenzyme + cofactor
Cofactors may be divided in to three groups
1) prosthetic group – e.g. porphyrin
2) Coenzyme – e.g. NAD, $NADP^{+}$
3) Metal activators – e.g. K^{+}, Mn^{2+}, Mg^{2+}, Zn^{2+} etc.

Properties of Enzymes
1) Catalytic property 2) Specificity 3) Heat destruction
4) Enzyme inhibitors 5) High molecular weight 6) Colloidal
7) Water soluble 8) Reversibility of action

Functions
1) To accelerate or retard or bring about reaction
2) Regulate reaction
3) To make possible the metabolic reactions
4) To facilitate reaction
5) To break down larger molecule to small molecule
6) To carry out flow of reaction smoothly.

Factors Affecting Enzyme Activity
1) pH 2) Temperature 3)Substrate concentration
4) Enzyme concentration 5) Concentration of any activator present
6) Concentration of any inhibitor present 7) Ionic strength
8) Redox potential 9) Concentration or reaction products.

Topic-20

Enzyme immobilization (inactivation) and its Industrial application in agro- industries

IMMOBILIZATION OF ENZYMES

Enzyme immobilization may be defined as confining the enzyme molecules to a distinct phase from the one in which the substrates and the products are present; this may be achieved by fixing the enzyme molecules to or within some suitable material. It is critical that the substrates and the products move freely in and out of the phase to which the enzyme molecules are confined. Immobilization of enzyme molecules does not necessarily render them immobile; in some methods of immobilization, *e.g.,* entrapment and membrane confinement, the enzyme molecules move freely within their phase, while in cases of adsorption and covalent bonding they are, in fact, immobile.

The materials used for immobilization of enzymes, called *carrier matrices,* are usually inert polymers or inorganic materials. The ideal carrier matrix has the following properties: (i) low cost, *(ii)* inertness, *(iii)* physical strength, *(iv)* stability, *(v)* regenerability after the useful lifetime of the immobilized enzyme, *(vi)* enhancement of enzyme specificity, *(vii)* reduction in product inhibition, *(viii)* a shift in the pH optimum for enzyme action to the desired value for the process, and *(ix)* reduction in microbial contamination and non-specific adsorption. Clearly, most matrices possess only some of the above features. Therefore, carrier matrix for the immobilization of an enzyme must be chosen with care keeping in view the properties and limitations of various matrices.

Methods of Immobilization

The various methods used for immobilization of enzymes may be grouped into the following four types: *(i)* adsorption, *(ii)* covalent bonding, *(iii)* entrapment, and *(iv)* membrane confinement. .

Adsorption. In case of *adsorption,* the enzyme molecules adhere to the surface of carrier matrix due to a combination of hydrophobic effects and the formation of several salt links per enzyme molecule. The binding of enzyme molecules to the carrier matrix is usually very strong, but it may be weakened during use by many factors, *e.g.*, addition of substrate, pH or ionic strength.

Covalent Binding. In this system the enzyme molecules are attached to the carrier matrix by formation of covalent bonds. As a result the strength formation occurs with the side chains of amino acids of the enzyme, their degree of reactivity being dependent on their charged status. Roughly the following relation is observed in reactivity:

$-S^- > -SH > -0^- > -NH_2 > -COO^- > -OH >> -NH_3^+$

Entrapment. In this approach, enzyme molecules are held or entrapped within

suitable gels or fibres and there may or may not be covalent bond formation between the enzyme molecules and the matrix. A non-covalent entrapment may be viewed as putting the enzyme molecule in a molecular cage just as a caged bird / animal. When covalent binding is also to be generated, the enzyme molecules are usually treated with a suitable reagent.

Membrane Confinement. Enzyme molecules, usually in an aqueous solution, may be confined within a semipermeable membrane which, ideally, allows a free movement in either direction to the substrates and products but does not permit the enzyme molecules to escape.

Effects of Immobilization on Enzyme

Often kinetic behaviour of an immobilized enzyme may differ significantly from that of its free molecules. Different enzymes respond differently to the same immobilization protocol. Therefore, a suitable immobilization protocol has to be worked out for a given enzyme. The effects on enzyme kinetics *(i.e.* activity) may be due to the influence of matrix *per se* or due to conformational changes in the enzyme molecules induced by the procedure of immobilization.

Advantage of Immobilization

Enzymes are costly items, and can be used repeatedly only if they can be recovered from the reaction mixtures. Immobilization permits their repeated use since such enzyme preparations can be easily separated from the reaction system.

1. Immobilized enzymes can be used in non-aqueous systems as well, which may be highly desirable in some cases.
2. Continuous production systems can be used, which is not possible with free enzymes.
3. Thermostability of some enzymes may be increased. For example, glucose isomerase denatures at 45°C in solution, but is stable for about 1 yr even at 65°C when suitably immobilized.
4. Recovery of enzyme may also reduce effluent handling problems.
5. Enzymes can be used at much higher concentrations than free enzyme.

<u>Uses of enzymes in solution</u>

Enzymes have a wide variety of applications in industry, medicine research etc. Some of the important applications are briefly discussed under the following headings: (i) uses of enzymes in solution, *(ii)* use of bi-phasic systems, *(iii)* uses of immobilized enzymes, and (iv) biosensors. The various uses of enzymes in solution are briefly described below.

I. Detergents

Detergents represent the largest industrial application of enzymes amounting to 25-30 % of the total sales of enzymes. The enzymes used in detergents must be cost effective, safe to use and be able to perform the task in

the presence of anionic and non-ionic detergents, soaps, oxidants etc. at pH between 8 and 10.5. Enzymes constitute only 0.4 - 0.8 % crude enzyme by weight (about 1 % by cost) of detergents. The chief enzymes used are proteases, α-amylase and, sometimes, cellulase.

1. Proteases are used to digest away proteins present in blood stains, milk, grass etc. and also in association with dirt; therefore, they help in removal of dirt as well. Only serine proteases are suitable for use in detergents. These enzymes are produced by *Bacillus licheniformis* and *Bacillus amyloliquefaciens.* Proteases are packed inside dust-free granules coated with wax materials made from Paraffin oil or PEG plus hydrophilic binders; the granules disperse in wash releasing the enzyme. This strategy protects users from hypersensitivity to the enzymes.

2. a-Amylase is used to digest away starch present in association with dirt and stains; they are produced by *B. licheniformis.*

3. Cellulases, produced by fungi, are used for washing cotton fabrics. The enzyme digests away the small fibers raised from the fabric without damaging the major fibers of the fabric. This restores the fabric to 'as new' condition, and also removes soil particles by digesting the associated cellulose.

4. Lipases suitable for detergent use have been identified and are used for digestion lipids present in stains and/or dirt.

II. Leather Industry

Alkaline proteases (0.1-1 % w/w) are used to remove hair from hides; this is safer and more pleasant than the traditional method using sodium sulphide. Dehaired hides are processed or bated often using pancreatic enzymes to increase their suppleness and softness in appearance. Bating is necessary for the production of soft leather clothing.

III. Wool Industries

Wool fibers are covered with overlapping scales pointing towards the tip; this favours problem is successfully overcome by a partial digestion of the scales by papain (protease); this process also gives the wool a silky appearance and adds to its value. However, the process is no more in use due to economic reasons (mainly high cost of papain), but is likely to be initiated again with the availability of cheaper enzymes.

IV. Food, Dairy, Juice and Beverages Industries

Several processes in the production of food, beverages etc. utilize enzymes; e.g. production of glucose syrup, maltose syrup and sucrose industry for preparation of invert syrup.

V. *Use in Medicine*

Enzyme applications in medicine are as extensive as in industry. Pancreatic enzymes have been used in digestive disorders since nineteenth century. Most enzymes are used extracellularly for *(i)* topical applications, *e.g.*, collagenase, *(ii)* removal of toxic substances, *e.g.*, rhodonase, or in *(iii)* disorders within blood circulation system, *e.g.,* streptokinase, urokinase etc. The enzyme preparations must be of high purity and free from unwanted

contamination; therefore, they are generally from animal sources and very costly. For example, urokinase is isolated from human urine and costs nearly $ *200/mg;* the annual market for this enzyme is nearly.. $150 million.

Enzymes have a major potential application in treatment of cancer, *e.g.* asparagenase in the treatment of lymphocytic leukemia. Tumour cells are unable to synthesize L-asparagine due to an enzyme deficiency, and obtain this amino acid from body fluids. Asparaginase drastically reduces the levels of free L-asparagine in the food stream, creating starvation in tumour cells for this amino acid; normal cell are not affected since they can synthesize L-asparagene. Asparaginase is injected intravenously, slows half-life of about 1 day (in dog), and may lead to complete recovery in 60% of the cases.

VI. Aspartame Synthesis

Aspartame is a dipeptide containing one residue each of L-aspartic acid and methyl ester of L-phenylalanine. It is 180 times more sweet than sucrose, and is used as low level amino group is protected by a reaction with, usually, benzyl chloroformate) and methyl ester of L-phenyalanine by the protease thermolysin. D-phenylalanine methyl ester is also added in a quantity equal to that of the L-isomer; the D-isomer forms an addition complex with aspartame which forms a precipitate. This removes aspartame from the reaction mixture and gives high yields at concentrations above 1 M. Later, aspartate is recovered from the precipitate by suitably changing the pH, and finally the benzyl chloroformate (attached to the amino group of L-aspartic acid) is removed by a simple hydrogenation process.

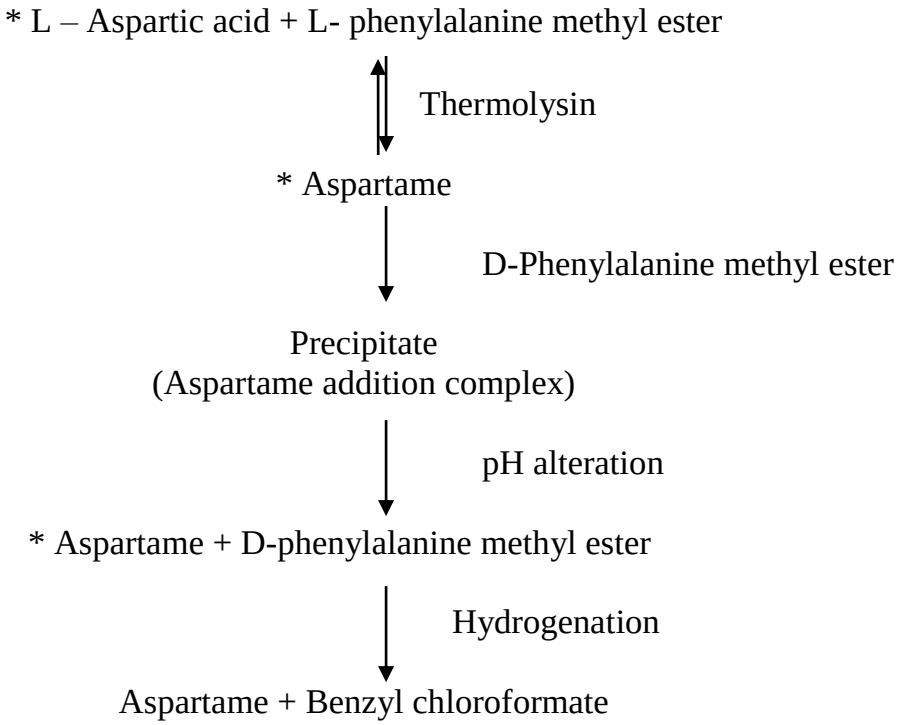


Fig. Synthesis of aspartame by thermolysin. * The amino group of L-aspartic acid is protected by a reaction with benzyl chloroformate.

LESSON PLAN – 21

Topics : Vitamins: Definition, classification, source, biological functions, deficiency disorders, vitamins with enzyme.

Definition of Vitamins

Vitamins are defined as organic compounds required in minute amounts for catalytic and regulatory functions in cell metabolism in absence of which certain deficiency diseases occur.

Classification

1) Fat soluble - Vit. A,D,E,K
2) Water soluble - Vit. B complex, Vit C.

Role as coenzymes in different biochemical reactions.

1) Oxidation reduction
2) Transfer of group.
3) Crboxylation
4) Transamination
5) Decarboxylation

Coenzyme derivatives of watersoluble vitamins and their function.

Sr. No.	Vitamin	Coenzyme derivatives	Functions Performed
1	Vit B_1 Thiamin	TPP	Aldehyde group transfcr
2	Vit B_2 Riboflavin	FMN FAD	Hydrogen group transfer Electron transfer in Redox reaction
3	Vit B_3 Pantothenic acid	CoA	Acyl group transfer
4	Vit B_5 Niacin	NAD, NADP	hydrogen group transfer
5	VitB_6 Pyridoxine	PP	Amino group lransfer
6	VitB_7 Biotin	Biocytin	Carboxyl group transfer i.e. CO_2 fixing
7	I Vit B_9 Folic acid	THFA	One carbon group transfer
8	Vit. B_{12} Cynocobalamin	Deoxyadenosyl cobalamin	Cofactor in hydroxylations

Vitamins, their sources, metabolic functions and disorders (Explain fat soluble and water soluble vitamins)

LESSON PLAN – 22- 24

Topic : Carbohydrate metabolism - Anabolism, catabolism, Glycolysis, pentosephosphate pathway (PPP), TCA cycle.

General introduction to metabolism

1) Anabolism - Building up phase, process of synthesizing complex compounds requires input or energy.

2) Catabolism Process of decomposition of breakdown or complex substances like lipids, carbohydrates, proteins for release of energy. ATP storage form of energy.

Carbohydrate metabolism

Glycogenesis - transformation of sugar to glycogen.
Glycogenolysis - Enzymatic breakdown of glycogen to glucose
Glyconeogenesis - Synthesis of glucose from non-carbohydrate material.

Major pathways of glucose utilisation in cells of higher plants.

1) Glucose may be stored as polysaccharides or sucrose
2) Oxidized to 3 carbon compound (pyruate) via-glycolysis.
3) Oxidized to pentose via pentose phosphate pathway.
Three major inter related pathways of carbohydrate metabolism.

1) *Glycolysis or EMP pathway* - Anaerobic conversion of glucose to pyruvic acid. Sequence of reaction that can operate without oxygen. Conversion of glucose to pyruvic acid and then to either ethyl alcohol or lactic acid.

Explain sequence of reaction of glycolysis

Reversible reactions except conversion of fructose 1-6 diphosphate to fructose - 6- phosphate. Yield 8 mole of ATP. 1 mole of glucose gives 2 moles of pyruvic acid.

II) Kreb's cycle or TCA cycle or citric acid cycle or aerobic oxidation of pyruvic acid - oxygen requiring continuation of glycolytic pathway under anaerobic condition pyruvic acid is converted into lactic acid but under aerobic conditions. Pyruvic acid is oxidized to CO_2 and H_2O through T.C.A cycle. Acetyl coenzyme A is the link between EMP pathway and Kreb's Cycle, common channel for product of glycolysis, ultimate oxidation of fatty acids and carbon skeleton of many amino acids.

Explain sequence of reactions in TCA cycle

Total energy out put from glucose - One mole of glucose gives 38 moles of ATP (8 ATP from anaerobic glycolysis + 30 moles from TCA cycle).

III) Pentose phosphate pathway or phosphogluconate pathway or Hexose monophosphate shunt or Warburg-Dicken's pathway.

Major function of this pathway

1) It acts as a source of pentoses for nucleotides synthesis i.e. ultimately for nucleic acid synthesis.
2) It is considered the alternate route for metabolism of glucose.

Explain sequence of reactions of pentose phosphate pathway

3 phospholglyceraldehyde and fructose -6- phosphate the end product of this pathway enters EMP pathway. Phosphogluconate is a key intermediate produced during the reactions.

LESSION PLAN – 25-26

Topic : Phosphorylation - Definition, cyclic, non cyclic, oxidative and substrate level phosphorylation.

Definition of phosphorylation:

Formation of a phosphate derivative of a biomolecule, usually by enzymatic transfer of a phosphate group from ATP.

Types of Phosphorylation:

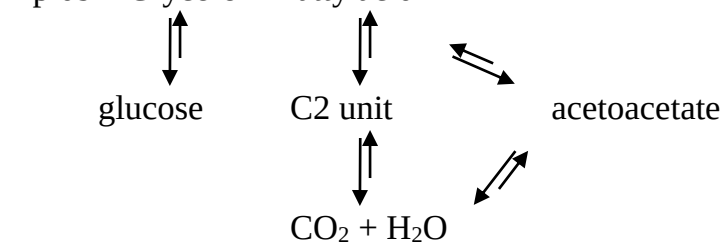
1) Photophosphorylation or photosynthetic phosphorylation.
   a) Cyclic photophosphorylation
   b) Noncyclic photophosphorylation
2) Substrate level photophosphorylation.
3) Oxidative phosphorylation.

1) Photophosphorylation - The enzymatic formation of ATP from ADP coupled to the light dependent transfer of electrons in photosynthetic cells.
   a) Cyclic photophosphorylation - ATP synthesis driven by cyclic electron flow through photosystem I. (explain with diagram)
   b) Non cyclic photophosphorylation - The expelled electrons do not return to the chloropylhyll, (explain with diagram).
2) Substrate level phosphorylation - Phosphorylated substrates donate inorganic phosphate to produce ATP (Non oxidative)
3) Oxidative phosphorylation - (linked to electron transport). The enzymatic phosphorylation of ADP to A TP coupled to electron transfer, from a substrate to molecular oxygen.

LESSON PLAN – 27

Topic : Lipid metabolism - Beta oxidation of fatty acid

Lipid metabolism is summarized below:



Beta oxidation of fatty acid

The degradation of fatty acid proceeds step wise by series of reactions which remove 2 carbon atoms at a time from the carboxyl end of the carbon chain. This catabolic scheme or mechanism is known as Beta oxidation which was proposed by Knoop in 1905.
This process takes place in 5 steps under control of different enzymes.

Explain sequence of reaction In Beta Oxidation

Steps in Beta-oxidation of fatty acids
1) Activation of fatty acids
2) Formation of unsaturated acyl-CoA
3) Formation of Beta hydroxy Acyl CoA
4) Formation of Beta-keto acyl CoA
5) Thiolytlc cleavage of Acyl CoA
Acyl CoA enter in citric acid cycle and oxidised to CO_2 and H_2O and produce ATP molecules.

Energy from oxidation of fatty acid.

Ex - palmitic acid (16 carbon) yield 8 molecules of acetate and run through the Beta oxidation cycle 7 times. Oxidation of a carbon (one cycle) yield 5 ATP (7 x 5 = 35 ATP) and 8 molecules of acetate where oxidized in TCA will produce (8 x 12 = 96). Total ATP = 35 + 96 = 131. Loss of 2 energy rich phosphate at activation step hence 131-2 = 129 ATP net gained.

LESSON PLAN – 28-29

Topic : Biosynthesis – Carbohydrates, Lipids, proteins and nucleic acids.

i) Carbohydrates : Pathways of glycogen and glucose synthesis from non carbohydrate source i.e. glycogenesis and gluconeogenesis.

ii) Biosynthesis of lipid (3 steps)
1) Synthesis of fatty acid
2) Synthesis of Glycerol
3) Condensation of fatty acids and glycerol

Steps in synthesis of lipids

i) Synthesis of malonyl-CoA from acetyl-CoA
ii) Structure and organisation of fatty acid synthestase system
iii) Regulation of fatty acid synthesis.
iv) Hormonal regulation of triacyl glycerol
v) Biosynthesis of phosphoglycerides

Topic : Protein metabolism – General reactions of metabolism of amino acids. Transmination, deamination, decarboxylation, biosynthesis of proteins.

General reactions of metabolism of amino acids.

1) Transamination - Enzymatic transfer of an amino group from an α amino acid to an (α) Keto amino acid.

2) Deamination - The enzymatic removal of amino groups from biomolecules such as amino acids or nucleotides.

3) Decarboxylation - Removal of CO_2 from the carboxylic group and convert the amino acid to its corresponding amines.

<u>Biosynthesis of proteins</u>

Three steps are involved in biosynthesis of protein.
1) Replication - Flow of genetic information from DNA. Synthesis of DNA
2) Transcription - Information contained in DNA is copied by base pairing to form complementary ribo-nucleotides form RNA. Synthesis of RNA.
3) Translation - Information contained on mRNA directs the ordered polymerization of specific amino acids to form protein.
Thus DNA makes RNA and RNA makes protein.

1) Replication and DNA Biosynthesis: Free deoxyribo-nucleotides are assembled linearly to form an identical sequence or replication of original DNA structure for hereditary transmission.
a] Initiation - Unwinding proteins are essential for initiation and continuation

of replication which separate the DNA strand for enzyme polymarise to function.

b] Elongation - Deoxyribonucleotide are properly positioned elongation will continue untill 500 to 1000 deoxyribonucleotide residues arc added to form doughter strands and RNA-DNA fragment (called Okazaki fragments).

c) Termination : As 3 OH terminus approach 5' PPP terminus 3 events occur - excision of RNA, filling of gaps with deoxyribonucleotide (done by DNA polymerase) and fusion of DNA fragments i.e. (3'OH terminus with 5' PPP terminus by enzyme DNA ligase.

2) Transcription and RNA Biosynthesis : Process of information flow from DNA to RNA. DNA base pair with rRNA, tRNA, mRNA form DNA-RNA hybrid (Okazaki fragment), occur only when region on DNA are complementary to reaction in RNA e.g. A-T-T-C-C in the DNA pairs with U-A-A-C-G in RNA. The RNA formed has a composition and sequence complimentary to that of DNA. RNA synthesis is copying reaction, reaction similar to DNA synthesis and proceed by base pairing A to T, G to C and U to A.

The synthesized RNA undergoes modifications in cytoplasm of prokaryotic cell to reduce the length of RNA to form ribosomal, messenger and transfer RNAs.

3) Translation and protein biosynthesis

a) Activation - Activation of amino acids by activating enzymes making use of ATP energy to form amino acyl-tRNA. Each amino acid has specific activation enzyme and t-RNA (to carry it at site of synthesis). (Code sequence of nitrogenous base in the DNA molecule constitutes the code which determines the order in which amino acids are joined to form the protein molecule, sequence of base of mRNA. Amino acid code 3 adjacent nucleotide residues on mRNA out of 64 triplet codon 61 codons encode amino acids and 3 are tenninating codons. Ribonsome's site of translation is ribonucleoprotein rRNA serves as structural polymer holding multiprotein particle in compact configuration).

b) Initiation: Binding of 30S subunit of ribonsome to mRNA in presence of IF3 (initiation factor). Next bindings of tMET-tRNA to 305-mRNA IF3 complex of 30S-mRNA. tMET-tRNA-GTP complex and release of IF3. next GTP hydrolysed and 50S subunit of ribosome combine with complex to form 70S complex containing $_t$MET-tRNA on P site. Codon AUG on mRNA.

c) Elongation - Stage 1) New acyl-tRNA bound to site A Stage. 2) Formation of peptide bond-peptidyl moiety of tRNA on P site. Stage 3) Translocation process. Shift of new peptidyl tRNA from A site to P site. Shift of ribose to next carbon on mRNA.

d) Termination - 2 event occur

i) The recognition of termination signal in the mRNA, Terminating codon VAA, UAG and UGA (nonsense codons).

ii) The hydrolysis of peptidyl tRNA linkage to release protein.
70S ribosome dissociate from mRNA into 30S and 50 S subunit to enter the protein synthesis.

LESSON PLAN – 30

Topic : Integration of carbohydrate, protein and lipid metabolism.

The metabolic process involving carbohydrate, protein and lipid can be divided in to three stages.

1st stage - Stage of hydrolysis to simple units.

2nd stage - Preparatory stage

3rd stage - Oxidative stage, citric acid cycle or the aerobic final pathway of metabolism

Give integration of metabolism of carbohydrates protein and lipids (Book A text Book of Biochemistry by AVSS Rama Rao)

LESSON PLAN – 31-33

Topic - Glycosides, tannins, lignins Gums and musilages: Defination, classification, properties and their physiological roles and application in food and pharmaceutical industries.

Glycosides: Definition: Chemical Nature : They are organic compound in which there is usually a semiacetal linkage between the reducing group of a sugar and an alcoholic or phenolic hydroxyl group of a non sugar compound called aglycon. Most glycosides are derived from D-glucose are called glucosides, β type in plants.

Classification: Four classes.
1) Cynophoric glycoside or cyanogenic glycoside
2) Mustard oil glycoside or Glucosinolates
3) Saponins
4) Phenolic glycosides.

1) Cynophoric - Yield HCN on hydrolysis e.g. Amygdalin in bitter almond; α-peachs and plums and Dhurin in young seedling of sorghum plant.

2) Mustard oil glycosides : Yield isothiocyanates (-NCS) on hydrolysis e.g. sinigrin in black mustard; sinalbin in white mustard.

3) Saponins: Have ability to form colloidal solution in water which gives a soapy foam and on hydrolysis yield variety of sugars. e.g. Digitalis and Strophanthus, Nimbidin.

4) Phenolic glycosides - aglycon of this class is aromatic compound containing phenolic group e.g. arbutin, salicin, indican.

Occurrence: Widely occur in roots, barks, fruits, to small extent in leaves.

Properties: Crystalline solid, colourless with bitter taste, soluble in water & org. solvents except ether, stable, do not show reducing property like glucose; on hydrolysis yield sugar and aglye (nonsugar).

Physiological Role: Serve as reserve food material, means for removal of toxic substances inhibit disease producing microorganisms, repel harmful insects and animals. In certain cases some insects are attracted which may help in pollination, act as protective agent against wound

References:

Singh and Singh (1966). Agril Chemistry National book house, Agra, pp.16-41.
Miller, L.P (1973). Phytochemistry, Vol.1 Van Nostrand Reinhold Co., New York, 211-248, 271- 296, 297-376.

Tannins (Tannic Acid) - High molecular weight (between 500-3000) compound containing sufficiently large number of phenolic hydroxyls or other suitable groups to enable it to form effective cross linkage between proteins and other macro molecules is called tannins.

Occurrence:

1 In plant parts-bark, wood, leaves and fruits, maximum in galls.
2 Indian berry Oke, tea leaves, bark of acacia

Chemical Nature

1. Complex derivative of gallic acid and are poly hydroxy phenols
2. Upon hydrolysis yield gallic acid, digallic acid + sugar (gl)

Give chemical structure of gallic acid

Properties:

1. Lusterous, faintly yellowish, amorphous, powder, spongy mass.
2. Darkens when exposed to air, odourless.
3. It decomposes at $21^{O}C$
4. Strong Astringent taste
5. PPT glue, protein, alkaloid
6. Soluble in boiling water, alcohol and acetone
7. Tannins form blue colour with ferrous sulphate later on converted to blue black colour.

Tannic + $FeSO_4$ → Ferrous tannate (Blue) $\xrightarrow{\text{Oxidation}}$ Ferric tannate (Blue, black)

Classification of Tannins : According to their behaviour on dry distillation into 2 groups

1. Condensed tannins - are formed by process of polymerisation of flavonols.

a. Catechin polymer-e.g. do not contain glucose and ester bonds. catechin, epigall ocatechin.

b. Diol condensed tannins- e.g. Teracacidin, Melaccidin

2. Hydrolysable tannins- A hydroxy acid is esterified with glucose These tannins are hydrolysed to form gallotannic acid (pyrogallol)

a. Gallotannic acid hydrolysis gallic acid e.g. Acertannin, sumac tannin.
b. Ellagi tannin hydrolysis Ellagic acid e.g. Jugalin tannin, corigolagin tannin.

Physiological Role:

1. It inhibits the enzymatic activity in plant in dead cells.
2. As reserve food material and means of removal of toxic compounds.
3. It takes part in the formation of pigments.
4. Tannins may serve as a protective agent for preventing the germination and growth of pathogenic fungi or organism in plants.

Uses:

1. Tannins are used in leather industries (Preparation of leather from raw hids)
2. Preparation of paints and dyes, paper ink.

Lignins :

Definition : It may be defined as incrusting material of the plant which is built up mainly of phenylpropane building blocks. It is a polymeric non-carbohydrate material. It is woody part of plant called as lignin and combination with cellulose is called ligno-cellulose.

Occurrence:

1. Lignin occur in woody tissues of plants.
2. Occurs only in secondary cell wall material In wood : 25-40%, straw : 15-25%.

Chemical Nature:

1. Lignin mostly consists of phenyl propane ring i.e. C_6-C_3 of the type represented by coniferyl alcohol, p-hydroxy cinnamyl alcohol and cinapyl alcohol.
2. It also contains large proportion of aromatic ring, with high content of methyoxy group (OCH) e.g. Syringaldehyde e.g., Vaxillin

Give chemical structure of syringaldehyle and Vaxillin)

Properties:

1. Amorphous and high molecular weight with, yellow brown colour.
2. Soluble in hot alkali and bisulfite and readily condensed with phenol and thiol compounds, unhydrolyzable by acid.
3. Insoluble in water and all organic solvents
4. On partial oxidation yield aldehydes and acids

Physiological Role:

1. It reduces the digestibility of feeding stuff.
2. It imparts biological strength to cellulose.
3. Affects the hydrophylic bonding components of middle lamella and of primary cell wall.
4. Cementing and anchoring cellulose fibre together and to protect cellulose from chemical, physical and biological attack.

Uses:
I. Used in paper industries
2. Preparation of hard binding and plywood.

Reference:
Miller (1973). Phytochemistry Vol. 1

Topic: Gums and Mucilage's

Gums: It is an exudation product of the plant and they are complex polysaccharides and amorphous, containing methyl pentose, hexoses and uronic acid residues joined by glycosidic linkage. Ex: gum acacia, plum gum, and guar gum.

Occurrence : Gums occur as exudate from the bark, leaves, stem and wounded part of plant as metallic salt of Na, K and Ca.

Chemical Nature:
1) Neutral salt of complex polysaccharide and branched chain nucleus of uronic acid (gluconic acid) residues
2) On hydrolysis with boiling water and acid split gums into uronic acid and some sugars (xylose, arabinose, fructose, glucose).

Properties :
Acidic in reaction, swells in water, Amorphous, soluble in water, form gel, insoluble in organic solvents, form salt with basis like Ca, Na, K.

Physiological Role :
Gums absorb water greatly and hence they hold water in plant tissues.

Typical Gums:
1) Gum Arabic (Acacia)- exudate from bark of acacia
2) Damson Gum-from bark of Damson plum tree
3) Tragacanth Gum- from leguminous plants
4) Guar Gum - obtained from guar plant
5) Microbial Gum - from capsules of certain bacteria.

Uses : Used in large scale in medicine and wood industry, manufacture of chewing gums.
Mucilages : Mucilages are sulphuric acid esters of complex polysaccharides and water extraction products from seed coat, plant root hair, sea weeds etc. They contain galacturonic acid and some sugars.
e.g. Agar agar, alginic acid.

Occurrence: They occur in seed coat of certain plant species. e.g. Linseed, flax mustard etc. and some sea weeds

Chemical Nature:
1. They are chemically complex polysaccharides, consisting of branched chain structure of uronic acid (galacturonic acid) and some sugar residues.
2. Naturally combined with proteins and cellulose.
3. They appear to be sulphuric acid esters of galactose.

Properties :
Absorb large quantity of water, swell, colloidal in nature, do not form gel, soluble in organic solvent. .

Physiological role :
They absorb large quantity of water and hold water in plant tissue. They help to retain moisture and used for seed germination.

Typical Mucilages :
1. AGAR - AGAR.
   a) It is prepared from various species of Gelidium, Asiatic sea weeds and red algae by extracting with water.
   b) It is chemically composed of sulphuric ester oflinear galactan.

2. ALGINIC ACID (Sodium alginate):
   a) It is extracted from sea weeds (Macrocystis, Laminaria and fucus) with Na_2CO_3.
   b) It contains polyuronic acid. On hydrolysis gives cellulose, uronic acid, mannuronic acid.

Uses : As demulcents, laxative, emulsifying agent, adhesive; useful in medicine and food industry.

LESSON PLAN – 34-36

Topic : Secondary metabolites--alkaloids, terpenoids - Definition, classification, properties and their physiological roles and application in food and pharmaceutical industries

ALKALOIDS:

Definition : Alkaloids are basic N containing heterocyclic compounds derived from higher plants often having marked physiological activity.

Occurrence : Occurs in flowers, leaves, stem, bark, roots, fruits as a salt of formic, malic, tartaric, citric, oxalic and acetic acids.

Chemical Nature : They are derivatives of heterocyclic Nitrogenous basic compounds such as pyridine, quinoline, isoquinoline and pyrrole.

Properties : Colourless, nonvolatile, crystalline (except nicotine) solids, insoluble in water but soluble in organic solvents, optically active, bitter in taste, poisonous to animals, form salts with acids, form precipitate with phospho-tungstic acid, phoshomolybdic acid, picric acid and mercuric iodie.

Physiological Role

They are active participants and not the end products of detoxification in plant metabolism. Adenine has important role in nucleic acid metabolism, certain purines act as growth regulating substances. Protect plant against insects. Some function as Coenzyme in oxidative process, act as stimulator, growth regulators, act as reservoir of protein synthesis.

Classification (According to heterocyclic nucleus)

Sr. No	Class	Example	Occurrence
1	Pyridine or Piperdine	Coniine piperine	Hemlock, piper longum
2	Pyrrolidine	Stachydrine	Stachys tuber hygrine
3	Pyrrolidine pyridine	Nicotine	Tobacco
4	Tropane	Cocaine Atropine	Coca leaves, Datura, plants of belladona sp.
5	Quinoline	Quinine	Cinchona bark
6	Isoquinoline	Papavarine Morphine	Opium poppy
7	Indole	Strychine Reserpine	Nux-vomica, Rauwolfia sp
8	Tropaline	Colchicine	Seeds and corms of colchicum

1) Pyridine: 1) Six membered ring

2) =CH replaced by -N

2) Pyrrolidine

Give chemical structure of pyridine and piperidine

2) – CH is replaced by N e.g. pyrrole and pyrrolidine

Give chemical structure of pyrrole and Pyrrolidine

3) Pyridine - Pyrrolldine. Nicotine – $C_{10}H_{14}N_2$
1. Combined two rings i.e. pyridine, pyrrolidine and having a side chain of methyl pyrrolidine, liquid at room temp.
2. On oxidation with chromic acid yields nicotinic acid

$$C_{10}H_{14}N_2 \xrightarrow{(0)} C_5H_4NCOOH$$

Nicotine Oxidation - Nicotinic acid

Give chemical structure of Nicotine

Properties

1. Colourless :
2. Oily liquid -B.P. $247^{O}C$
3. Turning brown in air due to oxidation
4. Tobacco like smell.
5. Alkali taste
6. Soluble in water and organic solvents
7. Highly toxic to animals
8. Stimulates the nervous system.

Uses : As an insecticide.

4. Tropane : Cocaine coca leaves ($C_{17}H_{21}O_4N$)
CHEMICAL NATURE : On hydrolysis yield ecgonine + methyl alcohol + benzoic acid.
ATROPINE : $C_{17}H_{23}O_3N$- found in Datura, on hydrolysis' yields tropine + Tropic acid

Give chemical structure of cocaine

5. Quinoline:
1. Benzene ring fused with pyridine ring and formed quinoline in α, β positions.
Quinine: $C_{20}H_{24}N_2O_2$. It occurs in bark of cinchona.

Chemical Nature: On vigorous oxidation with chromic acid yield quinnic acid.

Properties:

1. White solid crystal
2. Bitter taste
3. Soluble in organic solvent
4. It is a diacidic base, forming both neutral and acidic salts.

Give chemical structure of quinine

Uses:

1. Used for control of malaria,
2. Used as antipyretic.

6) Isoquinoline : It is made up of a benzene ring fused with a pyridine ring in different position.

Give chemical structure of Isoquinoline

Morphine : $C_{17}H_{19}O_3N$: It occurs in unripe fruits of opium poppy (10%). Upon oxidation yield isoquinoline and acid.

Properties :

1. Insoluble in water
2. Bitter taste
3. It affects central nervous system.

Uses :

1. It has soothing action
2. It is used as a Nacrotic drug, use in medicine.

7. Indole :

Union of benzene and pyrrole rings.

e.g. Strychnine, are obtained from dried seed of nux-vomica. It affects central nervous system.

Give chemical structure of Benzene, Pyrrole and Indole

Topic : Plant Pigments viz., Chlorophyll, carotenoids, flavonoids, anthocyanin, anthoxanthin.

Definition: Organic substances in plant that arc responsible in imparting various colours to plant parts are called plant pigments.

Occurrence : Protoplasm *of* the cells, chlorophyll, chloroplast of the cells.
Classification : Plant pigments are classified into two ways depending upon their solubility in fat *or* water.

i. Fat soluble. i. Chlorophyll ii. Carotenoid
a) Carotene - Light yellow, deep red, violet, purple colour pigment.
b) Xanthophyll - Yellow colour pigments
II. Water soluble i) Anthocyanin - red, blue, purple colour pigment.
ii) Anthoxanthin - yellow colour pigments.

I. Fat Soluble: Plant Pigments

A) Chlorophyll —> a) Chlorophyll' a' ($C_{55}H_{72}O_5Mg$) Ratio in plant is 3: 1
b) Chlorophyll 'b' ($C_{55}H_{70}O_6Mg$).

Properties

1. Black colour in solid state and greenish blue in colour in liquid state.
2. Helps in photosynthetic activities.
3. Occurs in protoplasm of the cell, strongly absorbs light in blue and red regions.

Properties

1. It is dark blue colour in solid state, absorbs light not effectively absorbed by chlorophyll 'a'.
2. Green colour in liquid state.
3. Occurs in blue algae.

All chlorophyll possess the property of fluorescence.

Chemical nature : Chlorophyll pigments : They are esters of complex dicarboxylic acid (phytol) and derived from porphyrin, a fully conjugated tetrapyrrole. It contains 4 pyrrole groups, 1 Mg atom in the centre and 2 ester groups. (Structure Mallette and Althouse book); Difference between chlorophyll a and b.

Physiological Role

1. Photosynthesis - conversion of solar energy to chemical energy (Chlorophyll).

B) Carotenoid :- Carotenoids are tetra terpene (polyene) consisting of eight

isoprenoid groups. Carotene ($C_{40}OH_{56}$)

1. Occurs in root of carrot.
2. It is soluble is ether.
3. Always associated with chlorophyll in the chloroplast.

Chemical Nature

1. It consists of hydrocarbon.
2. Carotene molecule consists of isoprene unit (C_5H_8)
3. On hydrolysis yield vit. A.
   $C_{40}H_{56} + 2H_2O$ hydrolysed $2C_{20}H_{29}OH$
   B-carotene vit. A

4. There are four isomers present in carotene

Name of isomer	% occurrence	On hydrolysis Vit. . A'
α - carotene	10% matured green leaves	1 molecule of Vit. 'A'
β - Carotene	90% matured green leaves	2 molecule of Vit. 'A'
γ - Carotene	Very little	1 atm of Vit. . A'
Lycopene	Red fruits	Not a precusor of Vit. A

(Structure of individual carotene not expected)

b) Xanthophyll ($C_{40}H_{56}O_2$) found in leaves, fruits, and flowers.

1. Yellow colour pigments, contains oxygen in various forms.
2. It is associated with carotene in plants

i) Lutein very important found in many plants
ii) Zeaxanthin - matured green leaves of maize and yellow seeds.
iii) Lycoxanthin - ye1low tomato fruit
iv) Cryptoxanthin- in maize and small quantity in citrus rind.

Physiological Role of Carotenoids

1. Protect the chlorophyll and sensitive enzymes in plant from destruction against harmful light radiation.
2. Helps in assimilation process
3. Carotene is a precursor of Vit. A.
4. It consists of terpene which is building block of essential oil.
5. Carotenoid occurs in photochemical reactions in plants.

II Water soluble plant pigments

Flavonoids : This group include red and yellow colour pigments which are water soluble substances present in vacuoles of cell sap of plants, glycosides, non sugar portion accounts' for colour.

Chemical Nature : Flavonoids are phenolic compounds having C_6, - C_3- C_6, skeleton.

a) Anthocyanin : They occur as glycosides involving sugars such as glucose, rhamnose, occasionally pentose.

Anthocyanin hydrolysis sugar + Non sugar (Anthocyanidin). They show different colours at different pH solution
1. Acid pH Red colour
2. Alkali pH - Blue colour
3. Neutral pH - Violet / Purple.

Chemical Nature :-

1. Anthocyanin are glycosides of benzopynylium nucleus
2. It also consists of sugar rhamnose, arabinose and glucose.
3. Anthocyanin nucleus consists of one pyrone ring and two benzene ring.
4. Nonsugary residue of anthocyanin is cal1ed anthocyanidin- derivatives of 2-phenylbenzopyrylium salts or derivatives of polyhydroxy flavylium.

(Give chemical structure of anthocyanine Nucleus)

b) Anthoxanthins

1. It is yellow colour pigment, found in flowers, fruits, leaves, bark, roots
2. It specially consists of rhamnose and glucose, freely soluble in cell sap
3. It is always associated with anthocyanin.

Chemical Nature:

1. Anthoxanthins nucleus consists of 2-phenyl benzo pyrone ring, include flavone and flavonol pigments, gycoside.
2. Non sugar residue of anthoxanthine is called as anthoxanthedin. Basic unit of aglycone portion or anthoxanthin is the gamma-pyrone.

Examples of Anthoxanthins
a. Flavone - Apigenin from the ivory white snapdragon
b. Xanthone - Gentisin from gentian root
c. Favonol - Quercetrin from bark of oak,
d. flavanones - Hesperidin from oranges.
e. Flavin - Riboflavin (vit B_2) distributed in plants and animals.

Physiological Role of Water Soluble Pigments

1. Flavonins attract the insects for pollination
2. Anthocyanin absorbs the heat and it increases the transpiration in cell.
3. Anthocyanin increases osmotic pressure in the cell.
4. Contribute to the food flavours a colours.

Topic: Essential Oils

Definition: Volatile oily products obtained often by steam distillation of freshly cut plant parts carrying the odour or flavour of the plants are known as essential oils.

Occurance: - Flowers, bark, leaves, wood, fruits.

Chemical Nature: are members of large groups of compounds derivatives of isoprene, 5 carbons (structure)

1. These compounds are not lipids but are oderiferous substances.
2. Heterogellous compounds.
3. Consists of monoterpencs, sesquiterpenes (hydrocarbons) numerous oxygenated compounds of all classes.
4. It consists complex mixtures of compounds like aldehydes, ketones, esters, ethers, phenyls, terpenes, and camphor and benzene derivatives. Some contain resins in solution are called oleoresins or balsams.

Properties

1. Pungent taste and odour
2. Nearly colourless when fresh.
3. They become darker after exposure to air.
4. They are optically active.
5. Soluble in alcohol, CS_2, CCl_4 petroleum ether and fatty acids.
6. Insoluble in acid and water.
7. No residue remain on cloth or paper

Physiological Role

1. Make plant part unpalatable to animals or parasitic insects.
2. In flowers, they serve to attract insects for pollination.
3. Repel the harmful insects.

Uses

1. It is used as medicine, acts as a stimulator or antiseptic local irritant, flavouring food, beverages.
2. It is used in preparation of perfumes.
3. Attractant and herrce help in cross pollination.

Classification of Essential Oils 4 Major groups

1. Aldehyde - Cinnamic aldehyde e.g Oil of cinnamon, oil of casia.
2. Ester - Methyl ester of salicylic acid e.g. oil of winter green
3. Ether - Aromatic ether e.g. oil of clove
4. Terpenes - i. Lemonine (+ dextrorotatory) *e.g. oil of lemon, oil of bitter orarge laevorotatory oil of pine needle.*
ii. Terpenoline cardamom oil.

Extraction of Essential Oils

Essential oils are extracted from plants by several methods as below:

1. Steam distillation.
2. Direct solvent extraction
3. The effleurage process

(Details of process is necessary)

Steam Distillation

The plant material prepared suitably is steam distilled. The oil if any is collected separately. The steam distillate is saturated with salt and extracted with purified solvents (petroleum and benzene). The combination and solvent extracts are dried and the solvent evaporated, some time through a fractionating column under reduced pressure to give the essential oils.

Direct Solvent Extraction

The plant material is extracted directly with solvents (ether and light petroleum) at room temperature. The filtered extract is evaporated under reduced pressure to yield the essential oils.

The Enfleurage Process

This method consists in leaving flower petals in contact with a fat preparation (an odourless mixture of lard and tallow) for several days. The fat is freed from the petals and stirred with absolute alcohol. The alcohol extract is evaporated in vacuum at O^{O} to give the essential oil.

Fractional Distillation

The terpene alcohols (eg. menthol) extracted by this method separation of aldehyde or ketone terpenes may make use of their reaction with carbonyl reagents such as sodium bisulphate, semicarbazide hydrochloride etc.

Important Essential Oils (structures not excepted)

1. **Peppermint :** from arial portion of mentha plant chief constituent terpene e.g. menthol
2. **Eucalyptus :** From eucalyptus tree - terpene.
3. **Terpentine** oil: From camphor - Terpene, pinene.
4. Oil of winter green :- Winter plant i.e. lower leaves of winter green plant and chief constituent is methyl salicylate.

1. Peppermint oil :- Menthol – $C_{10}H_{20}O$

Extracted by steam distillation from leaves. It is volatile white, crystalline.

Chemical Nature: It is fully saturated monocyclic mentha plant compound.

2. Limonene $C_{10}H_{16}$

Containing one ring and two double bonds.

Properties –

1. Insoluble in water
2. Readily volatile on steam
3. Pleasant odour.

3. *Eucalyptus oil*:- $C_{15}H_{26}O$: Extracted from leaves of eucalyptus tree

Chemical Nature :- The chief constituent is terpene. It is tertiary alcohol bicyclie, higher boiling fraction.

Properties

1. Colourless to pale yellow
2. Characteristic odour
3. Cooling taste
4. Miscible with alcohol.
5. Winter green oil: Extracted from the plant of winter green in which salicylic acid is used for preparation of asperin. It is also called methyl salicylate.

5. Terpene :- It is unsaturated hydrocarbon which includes both open chain and cyclic compounds e.g. Oil of lemon, oil of pine needle, terpentine oil from camphor.