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ONLINE COURSE WARE

SUBJECT NAME: CHEMISTRY OF FOOD

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Lecture 1

Introduction to different food groups (3, 5, 7 food groups) and importance of food chemistry:

A large variety of food is available to us which we include in our daily meals for taste and variety. Needless to say, we also get the nutrients our body requires from these foods. These nutrients are proteins, carbohydrates, fats, vitamins, and minerals. They are required in our body for energy, body, building, repair of tissues, protection from diseases and good health. Some foods are a good source of energy while others are good for body building or for protective functions. In order to ensure that our body gets everything that it requires we have to select from the foods available to us. For this purpose we classified the foods in different food groups.

1.1.Different food groups:

3 food groups: For the purpose of planning meals, the foods available can be broadly divided into three groups from the nutritional point of view. These are :

- Energy yielding foods
- Body building foods
- Protective foods
-

Functions	Major Nutrients	Examples
Body Building	Proteins	Milk, meat , chicken, Pulses
Energy Giving	Carbohydrates and Fats	Cereals, fats, sugar
Protective	Minerals and Vitamins	Fruits and vegetables

5 Food groups: All food can also be grouped into five food groups. In this system of food grouping similar food items are placed together. For example all cereals are similar in nutritive content and also similar in functions and all pulses are also similar in nutritive content. Similarly milk, egg and flesh foods are comparable and all oils, butter, ghee have similar nutrients.

- Cereals
- Pulses
- Milk, egg and flesh foods
- Fruits and Vegetables
- Fats and Sugars

7 food Groups:

- Green and yellow vegetables
- Oranges, grape fruit, tomato, raw cabbage
- Potatoes , other vegetables and fruits
- Milk and milk products
- Meat, poultry, fish and egg

- Bread, flour and cereals
- Butter or fortified margarine

Lecture 2

Water in foods and its properties: different types of moisture in food. Water activity: relationship between food's stability and water activity

1.2. Water in foods and its properties:

The water, chemical formula H₂O, is the major constituent of most foods. Although it brings any energy to food, its existence plays a very important role. It influences the structure, the appearance, taste of food and their susceptibility to degradation.

Water content of some foods:

Foods	Water content (%)
Beef	50 to 70
Chicken meat	74
Fish	65 to 81
Pears	80 to 85
Apples, peaches, oranges	85 to 90
Tomatoes, strawberries	90 to 95
Avocado, banana	74 to 80
Carrot, potato	80 to 90
Lettuce, lentils	90 to 95
Honey	20
Jam	28
Flour, rice	12
Milk powder	4

1.2.1. Functional properties of water in food:

- *Function solubilization (or dispersion):* Water in foods is the solvent of the hydrophilic constituents.
- *Function structure:* Water plays a key role in the pattern of food macromolecules, including proteins and carbohydrates. The water also determines the structure of certain constituents in the micelle. This applies, for example, casein in milk.
- *Mobilization function:* water, compared to other fluids, is the mobility factor of the response in food products.

1.2.2. Forms of Water in Foods:

The ease of water removal from foods depends on how it exists in the food product. The three states of water in food products are:

- **Free water:** This water retains its physical properties and thus acts as the dispersing agent for colloids and the solvent for salts.
- **Adsorbed water:** This water is held tightly or is occluded in cell walls or protoplasm and is held tightly to proteins.
- **Water of hydration:** This water is bound chemically, for example, lactose monohydrate; also some salts such as $\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$.

1.2.3. Water activity:

The a_w of a food or solution is the ratio of the water vapour pressure of the food or solution (p) to that of pure water (p_o) at the same temperature: - $a_w = p/p_o$

The water activity scale extends from 0 (bone dry) to 1.0 (pure water) but most foods have a water activity level in the range of 0.2 for very dry foods to 0.99 for moist fresh foods. Water activity is in practice usually measured as equilibrium relative humidity (ERH).

1.2.4. Relationship between foods stability and water activity:

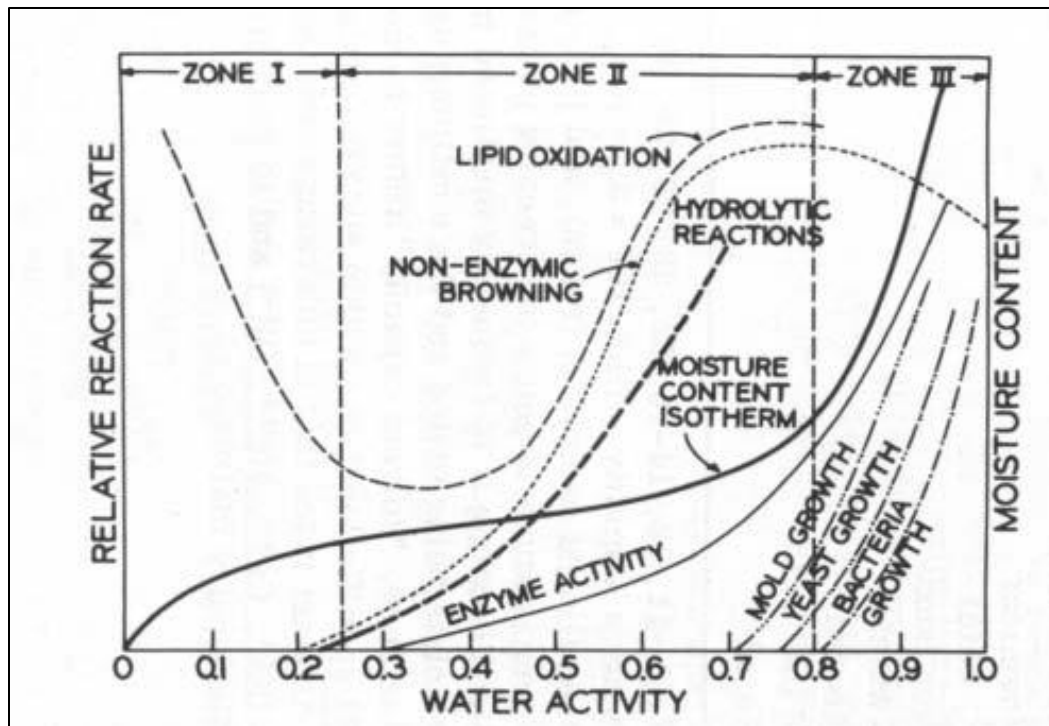
Water activity affects the shelf life, safety, texture, flavor, and smell of foods.

While temperature, pH and several other factors can influence if and how fast organisms will grow in a product, water activity may be the most important factor in controlling spoilage.

Most bacteria, for example, do not grow at water activities below 0.91, and most molds cease to grow at water activities below 0.80.

By measuring water activity, it is possible to predict which microorganisms will and will not be potential sources of spoilage.

Water activity--not water content--determines the lower limit of available water for microbial growth. In addition to influencing microbial spoilage, water activity can play a significant role in determining the activity of enzymes and vitamins in foods and can have a major impact their color, taste, and aroma.



Lecture 3

IMFs; Determination of moisture content, water absorption isotherm, drying curves

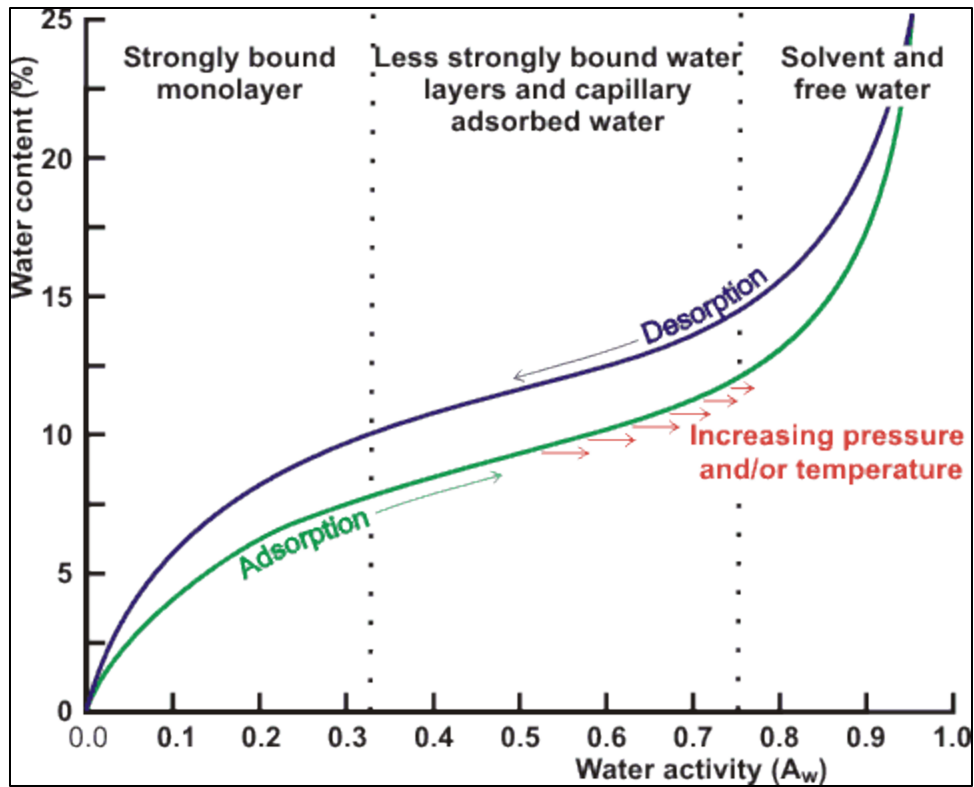
1.2.5. IMFs: INTERMEDIATE MOISTURE FOODS or IMFs are food product that has a water activity below that which is required for the growth of microorganisms; or a food containing unavailable water. IMF foods have water activity in B/w 0.6-0.9 or 10 to 50% moisture.

Purpose of intermediate moisture food:

- The purpose is to achieve a desirable water activity by the various ingredients so that food product maintain enough water for palatability and can be stored safely.
- Addition of preservatives provides the margin of safety against spoilage organisms
- *Staphylococcus aureus* is one of the organism of high concern which can tolerate aw as low as 0.83-0.86 under aerobic conditions.

Examples of IMFs: Jams, Jellies, Candies, Baked foods, Honey, Dried fruits and vegetables etc.

1.2.6. Water absorption isotherm: The relationship between water activity and moisture content at a given temperature is called the moisture sorption isotherm. This relationship is complex and unique for each product due to different interactions (colligative, capillary, and surface effects) between the water and the solid components at different moisture contents. An increase in a_w is almost always accompanied by an increase in the water content, but in a nonlinear fashion.

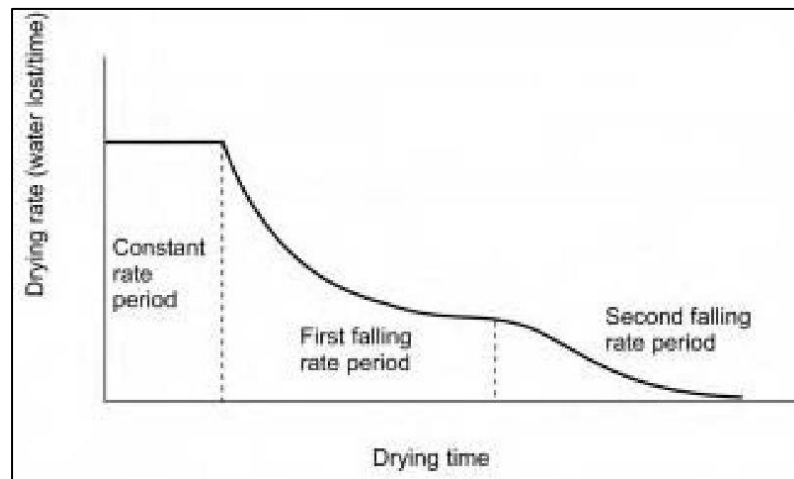


1.2.7. Glass Transition Temperature:

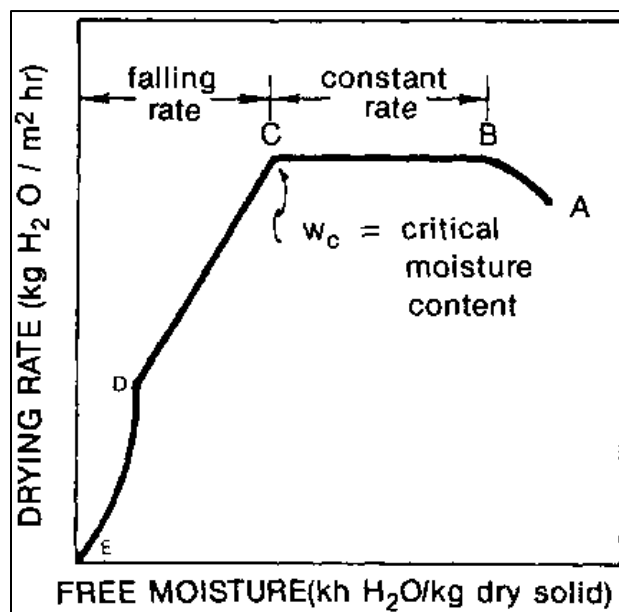
The glass transition -point is the temperature (T_g) at a given moisture content where a transition from a glassy stable amorphous solid state to a rubbery amorphous solid state can begin to take place. It has been used as indicator of food stability and to predict the behavior of foodstuffs. The glass transition temperature T_g varies according to the types of components and the water content of the food. Glassy foods have an amorphous metastable structure. However, when the water content or the temperature of a glassy material is increased, the material changes to a rubbery state. Therefore, the control of water content and temperature of glassy foods is of great importance in the process of food manufacture, transportation and preservation as well as storage.

1.2.8. Drying curve and Drying rate curve:

Drying curve:



Drying rate curve:



Critical moisture content: The moisture content at which the constant rate drying period ends and the falling rate drying period starts is called critical moisture content.

Equilibrium moisture content: The moisture contents of solid when it is in equilibrium with given partial pressure of vapour in gas phase is called as equilibrium moisture content.

Lecture 4

Carbohydrate: Sources of food carbohydrates; Classifications and examples with structures.

1.3. Carbohydrate: Sources of food carbohydrates; Classifications:

1.3.1. Definition: Carbohydrate is an organic molecule found in animals, plants etc., and composed of carbon, hydrogen and oxygen. Sometimes it is called hydrate of carbon.

Empirical Formula : $C_x (H_2O)_y$

1.3.2. Sources:

- Cereals
- Pulses
- Fruits and Vegetables
- Nuts and oilseeds
- Miscellaneous: Sugar, jiggery, honey, dates, skim milk powder etc.

1.3.3. Classification of Carbohydrates:

Carbohydrates can be classified in two ways:

I. Classification depending on their digestibility:

- **Available Carbohydrates:** The available carbohydrates, that is sugar plus starch, were defined as those that are digested and absorbed by the human small intestine and which are glucogenic.
- **Unavailable Carbohydrate:** The unavailable carbohydrates were defined as those that are not digested by the endogenous secretions of the human digestive tract. These are now generally referred to as dietary fiber.

II. Chemical classification of carbohydrates:

- Monosaccharides:** Monosaccharides are simple sugars, which possess a free ketone or aldehyde group. Being the simplest of sugars, they cannot be further hydrolyzed. Their chemical formula is $C_nH_{2n}O_n$ or $C_n(H_2O)_n$. Monosaccharides are classified into trioses, tetroses, pentoses, etc., and as ketoses or aldoses, depending on their ketone or aldehyde group.

Examples include glucose, fructose, galactose, glycerose, ribose, and ribulose.

- Oligosaccharides:** "Oligo" meaning few, oligosaccharides are sugars that break down into two to 10 molecules of monosaccharides when hydrolyzed.

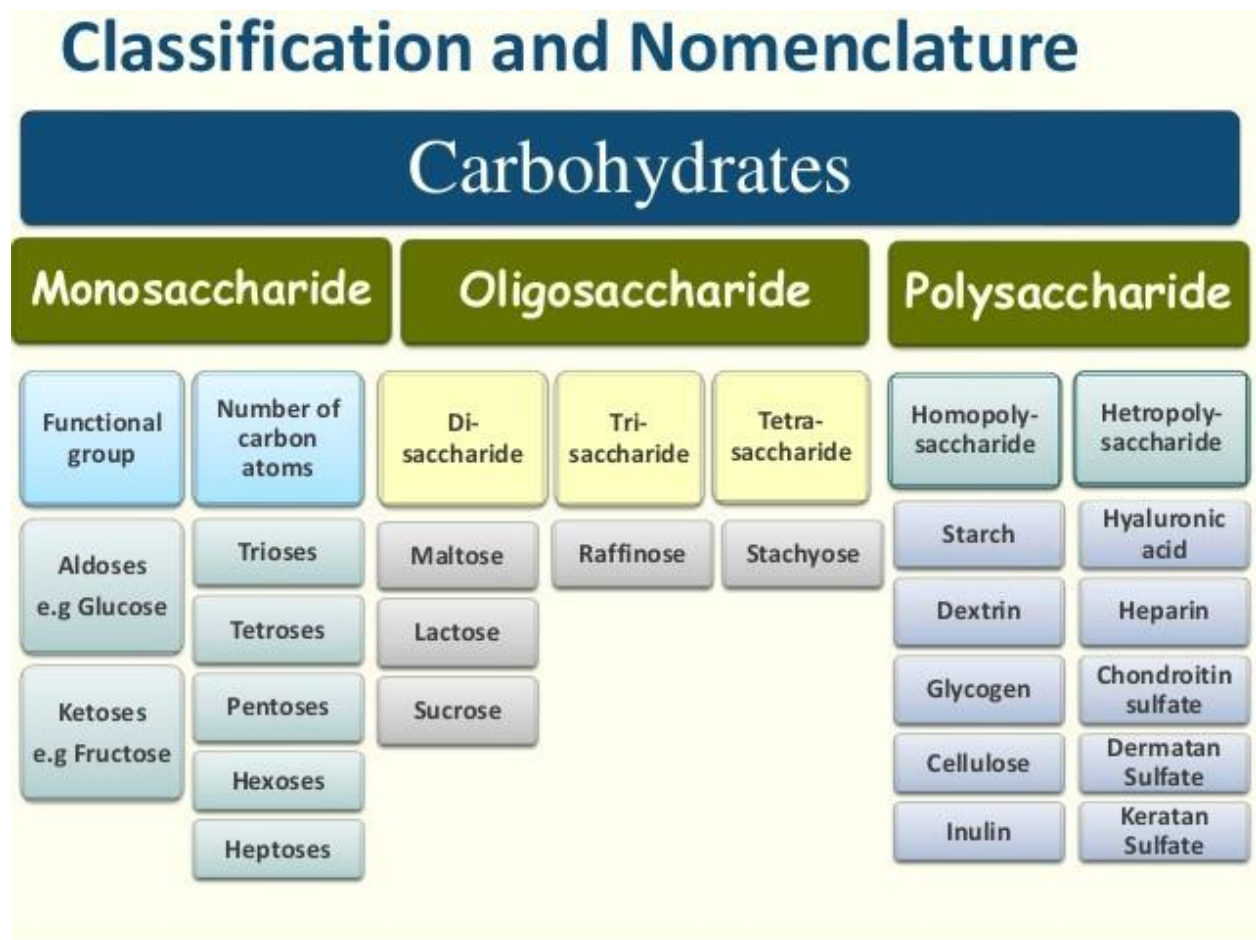
An oligosaccharide that yields two monosaccharide molecules on hydrolysis is a

disaccharide, while those that break down into three or four monosaccharides are called trisaccharides, tetrasaccharides, and so on. Disaccharides have a chemical formula of $C_n (H_2O)_{n-1}$ while trisaccharides and others are $C_n (H_2O)_{n-2}$, etc.

Oligosaccharide examples include sucrose, maltose, lactose, raffinose, and stachyose.

- iii. **Polysaccharides:** “Poly” meaning many, polysaccharides are compound molecules that yield more than ten monosaccharide molecules on hydrolysis. They are also classified depending on the type of molecules hydrolyzed. These include homopolysaccharides (with several monosaccharides of one type) or heteropolysaccharides (with different types of monosaccharides). $(C_6H_{10}O_5)_x$ is their chemical formula.

Polysaccharide examples include starch, cellulose, pectin, glycogen, inulin, and hyaluonic acid.



Note: Animal Polysaccharide: Glycogen

Lecture 5

Physico-chemical and functional properties: Monosaccharide: Physical properties of monosaccharide: isomerism, optical activity, muta-rotation; Chemical properties of monosaccharide: oxidation, reduction and Osazone test;

Functional Properties of Carbohydrates:

1.3.4. Physiological Functions:

I. Provide instant energy to the body: This appears to be the primary function of carbohydrates in the body.

Carbohydrates which we consume as food in the form of starch (ex: potato, bread), sucrose (ex: sugar, fruits) etc. get digested in the body to release glucose. This glucose after being absorbed into blood reaches all the body tissues and cells. There it gets metabolized to release energy in the form of ATP in the presence of oxygen inside the mitochondria. Thus energy is produced in the body due to breakdown of carbohydrates and it is the prime function of carbohydrates.

II. Reserve food for the body emergency: The excess glucose in the body is converted to glycogen in the liver and stored there for future needs like in starvation. Some of the glycogen is also reserved in muscles. In times of starvation, this glycogen converts back to provide energy.

III. Carbohydrates form other bio molecules: Carbohydrates in excess are converted into other bio-molecules of physiological importance like fats, by fatty acid synthesis reaction in the cell for storage in the body and use in times of starvation.

IV. Detoxification of the body by metabolism: Many drugs, toxic wastes in the body are metabolized for easy excretion in the body.

Some of these are water insoluble and hence they are difficult to be expelled in urine. The body converts them into glucouronyl conjugates using the glucouronyl moiety derived from carbohydrates.

A carbohydrate moiety like glucose combines with uronic acid to form glucuronate. These conjugates of insoluble substances with glucouronyls are more water-soluble and easily excreted from the body. Thus detoxification of physiological importance is carried out to some extent with carbohydrate derivatives.

V. As reaction intermediates or accessories: Carbohydrates participate as reaction intermediates in some vital reactions.

This function of carbohydrates is seen extensively in various cellular reactions. For example, Vitamin B2 i.e. Riboflavin has ribose sugar (4 carbon) a type of carbohydrate in its chemical structure and involved in vital reactions at cellular level. As such carbohydrates are constituents of many hormones, vitamins, enzymes etc.

VI. Constitute genetic material: Carbohydrates form a part of genetic material like DNA and RNA in the form of deoxyribose and ribose sugars. This as carbohydrates form heptulose sugars which are used to form ribose sugars (pseudo-heptulose pathway)

VII. They are constituents of all the cellular organelles like cell membrane, mitochondria, nucleus, endoplasmic reticulum etc. in one or other way to give structural integrity. They help make up the body mass by being included in all the parts of the cell and tissues. For example, in cell membranes, there are two constituents i.e. glycolipid layer and glycoprotein layer.

VIII. They form components of bio-molecules which have a key role in blood clotting, immunity, fertilization etc. Thus they take part in many physiological reaction.

IX. Transport of oxygen: Glucose is taken by red blood cells. These are the types of blood cells which lack mitochondria and other cell organelles required for production energy. In such case the ATP is produced by non-oxidative pathway (lactose pathway). This energy thus produced is necessary for hemoglobin to bind to oxygen molecules and aid in transfer of oxygen from lungs to the different tissues.

X. Aid in gut motility: Carbohydrates form fibrous material. When carbohydrates are digested, this material absorbs water in the guts, swells and increases the load. This load is useful to increase intestinal motility and expulsion of waste (feces). Thus carbohydrates help clear gut and prevent constipation.

1.3.5. Functions of Monosaccharaides:

Physical Properties:

1. Isomerization
2. Optical activity
3. Muta-rotation

Chemical Properties:

1. Oxidation reactions:

- Glucose oxidation with mild oxidizing agent
- Glucose oxidation with strong oxidizing agent
- Glucose oxidation at C-6 position
- Oxidation of galactose
- Oxidation of fructose

2. Reduction Reactions:

- Reduction reactions of glucose
- Reduction reactions of fructose

3. Cyanahydrin reaction

4. Osazone test

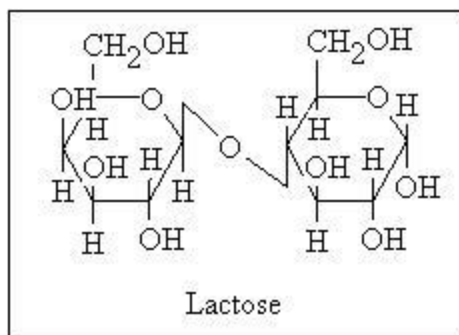
Lecture 6

Properties of disaccharides: Sucrose, Lactose etc. Polysaccharides: Chemistry and structure of homosachharides and heterosachharides.

Functions of Disaccharides:

1.3.6. Lactose:

Lactose is a disaccharide sugar composed of galactose and glucose that is found in milk and different dairy products. It has a formula of $C_{12}H_{22}O_{11}$ and the hydrate formula $C_{12}H_{22}O_{11} \cdot H_2O$. Lactose is a disaccharide derived from the condensation of galactose and glucose, which form a β -1:4 glycosidic linkage.

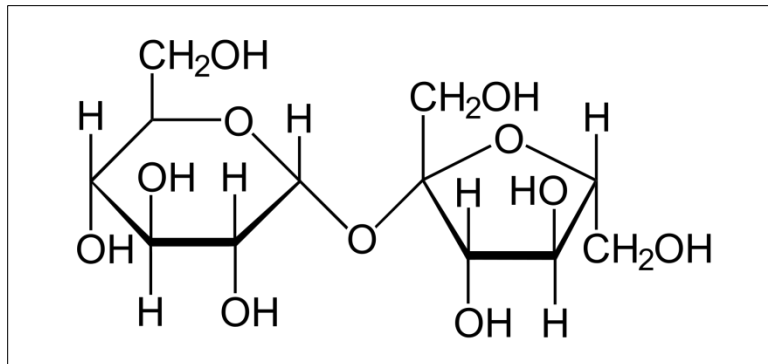


Properties of lactose:

- Hydrolysis of lactose
- Reduces Benedicts' reagent
- Reduces Barfoeds' reagent
- Molisch's test
- Mucic acid tes

1.3.7.Sucrose:

Sucrose is a common saccharide found in many plants and plant parts. Saccharose is an obsolete term for sugars in general, especially sucrose. The molecule is a disaccharide combination of the monosaccharaidesglucose and fructose with the formula $C_{12}H_{22}O_{11}$. In sucrose, the components glucose and fructose are linked via α -1:2 glycosidic linkage.

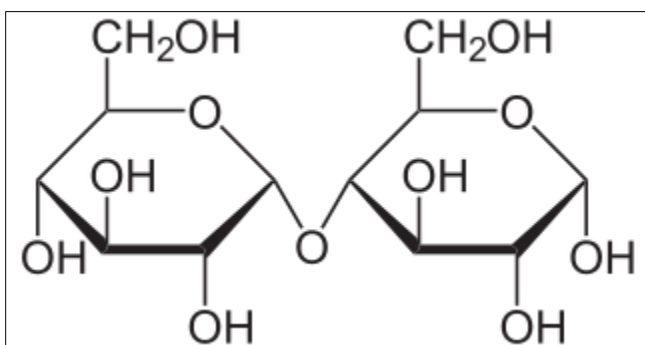


Properties of Sucrose:

- Hydrolysis of sucrose
- Invert sugar
- Molisch's test
- Caramelization

1.3.8.Maltose:

Maltose, also known as maltobiose or malt sugar, is a disaccharide formed from two units of glucose joined with a α -1:4 glycosidic linkage.



Properties:

- Fermentable sugar
- Hydrolysis of maltose
- Osazone test

Polysaccharides:

1.3.9. Classifications:

1. **Homoglycan:** A polysaccharide consisting of only one type of monosaccharide subunit (glucan).

2. **Heteroglycan:** Polysaccharides consisting of molecules of more than one sugar or sugar derivative are called heteropolysaccharides(heteroglycans).

Lecture 7

Starch: Structure, sources, properties (hydrolysis, gelatinization, retrogradation, dextrinisation, crystallization):

1.3.10.Starch:

Definition:

Starch is a polysaccharide and made of only glucose molecules. Hydrolysis of starch yield only glucose molecules. So starch is expressed as $(C_6H_{10}O_5)_n$. Starch molecules are widely distributed in mainly plant kingdom as well as vegetables and animals. Verities of sources of starch are potato, beet, various cereals like wheat, paddy, corn, maize etc. Starch molecules have about 14-19% of water, of which 10% is chemically bounded.

Mainly starch is composed of 12-20% of amylose, a water soluble portion and 80-90% of amylopectin, a water insoluble portion. Both these amylose and amylopectins are composed of glucose molecule and linked together through alpha 1:4 and alpha 1:6 glycosidic linkage.

Amylose: Amylose is long unbranched chain of glucose molecule linked together by alpha 1:4 glycosidic linkage. It's molecular weight is ranged from 10^5 to 10^6 . One molecule of amylose contains 500-5000 units of glucose. Amylose is water soluble. From the structure of amylose we can see, one end of this molecule bears nonreducing end another end bears the reducing end.

Amylopectin:It is insoluble in water. Amylopectin's molecular weight is 10^6 to 10^7 which is greater than amylose's molecular weight. One molecule of amylopectin contains 50,000-50,000,0 units of glucose. Amylopectin is a branched chain and each branch contains 20-30 glucose units linked by alpha 1:4 glycosidic linkage. The branches are linked together by alpha 1:6 glycosidic linkages.

Differences between amylose and amylopectin:

Amylose	Amylopectin
1. Amylose has a linear structure.	1. Amylopectin has a branched structure.
2. Amylose is water soluble.	2 amylopectin is water insoluble.
3. Amylose has less molecular weight than amylopectin. It is about 10^5 - 10^6 .	3. Amylopectin has more molecular weight than amylose. It is about 10^6 - 10^7 .
4. Amylose has good thickening power.	4. Amylopectin has less thickening power.
5. In amylose the glucose molecules are linked together with alpha 1:4 glycosidic linkage.	5. In amylopectin the glucose molecules are linked together with alpha 1:4 and alpha 1:6 glycosidic linkage.
6. It is able to form gel as it is water soluble.	6. It is unable to form gel as it is water insoluble.

Classifications of starch:

Starch can be classified into four categories.

1. Native starch or common starch or wild starch
2. **Waxy starch:** It contains mainly amylopectin. It possesses excess paste clarity, high hydrogen binding capacity, resistant to gel formation and retrogradation.
3. **Resistant starch:** Resistant starch is defined as the starch which escapes the enzymatic hydrolysis in small intestine and gets fermented in colon. It is present in fruits and vegetables.
4. **Modified starch:** It is prepared by physically, enzymatically or chemically treating the native starch. Thus changing the properties of native starch.

Starch may be modified:

- To increase their stability against excessive heat, acid, shear, cooling, freezing
- To lengthen or shorten gelatinization time
- To increase their viscostability

Some of the treatments producing Modified Starch:

1. Acid treated starch with inorganic acid (HCl)
2. Bleached starch by H₂O₂
3. Acetylated starch, esterification with acidic anhydride etc.

Applications of modified starch:

1. Pregelatinised starch is used to thicken instant dessert allowing the food to be thick with addition of cold water and milk.
2. Can use as fat substitute.
3. It is added to frozen product to prevent them from dipping at defrost state.
4. Acts as emulsifier for French dressing.
5. Oxidized starch increased the stickiness of batter.

Properties of starch:

1. Hydrolysis of starch:

- Acid hydrolysis:
- Enzymatic hydrolysis:

2. Gelatinization of starch: Gelatinization refers to the irreversible loss of the crystalline regions in starch granules that occur upon heating in the presence of water. This occurs when starch is treated with water, generally at high temperature. Swelling of starch granules under sufficiently high temperature and by applying good agitation, settlement of the swelt granules to the bottom portion of the mixture is known as gelatinization of starch. Temperature requirement is generally (80-100) °C and above.

Due to this phenomenon the starch molecules undergo swelling. The temperature at which the

swelling of starch granules starts is called gelatinization temperature.

The rate or extent of gelatinization depends upon the following factors:

- Size and shape of the starch granules
- Energy requirement for swelling of starch granules
- Influence of other ingredients in starch:
 - i. **Sugar:** This will decrease the extent of gelatinization
 - ii. **Acid:** It can reduce the thickness of hot starch paste and the firmness of the cold starch paste. Acid hydrolysis can convert the starch molecules in dextrin. As a result the extent of gelatinization decreases.
 - iii. **Fat and protein:** If fat or protein is added into the solution then, it will form a coat of layer around the starch molecules. As a result hydration can be delayed and development of viscosity will be lowered. So the rate of gelatinization decreases.
 - iv. **Agitation:** Slight agitation is necessary during starch gelatinization. But, higher degree of agitation can accelerate the rupture of the starch molecules. As a result viscosity will be decreased and the rate of gelatinization also decreased.

Changes during gelatinization of starch:

- i. Light transmission increases during the process of gelatinization.
- ii. Sensitivity of starch molecules towards enzyme attack increases. It increased the availability of starch for digestion.

3, Retrogradation of starch: It is commonly regarded as the normal progression of firming of starch gel at 0°C or lower. Generally it is found when starch molecules undergo any low temperature treatment. Starch is composed by amylose and amylopectin. Amylopectin has not been influenced by retrogradation but amylose fraction is influenced by this process. Retrogradation causes difficult to hydrolyse starch molecule through enzyme. Due to this process digestibility of starch reduces.

When bread stored under refrigerated condition, an undesirable texture change occurs due to retrogradation. It happens due to formation of some crystalline aggregates of amylose. Some hydrogen bonds which hold the gel together break and amylose molecules move around for forming new bonds. As the gel stales, amylose molecules rearranges in an orderly manner and for this reason some water releases and the gel causes some defect in the texture of the food.

Stale breads become soft when it is covered and reheated. But, when the stale bread is cooled down again an undesirable texture develops.

Retrogradation depends on the following factors:

- i. Size of the starch granules
- ii. shape of the starch granules
- iii. temperature of the gel formation
- iv. pH of the starch solution

- v. Presence of any other substances in the starch solution.
- vi. Concentration of the starch solution

4. Dextrinisation of starch: Dextrinisation is the chemical conversion of starch molecule into short chain starch molecules of variable length, known as dextrin. This process involves the application of high temperature (above 100°C) and the breakdown of one or more alpha 1:4 glycosidic linkage in the starch molecule. Dextrinisation is found when flour is browned during heating. Brown flour has lesser thickening ability than that of ordinary flour.

5. Crystallisation of Starch: Many polar organic solvents like nitroethane, nitropropane, nitrobenzene, methyl-ethyl ketone, pyridine, monohydroxy alcohol etc., form complexes with amylose in aqueous dispersion of starch. This looks like needle shaped crystals and it will be precipitated out. Almost quantitatively all the amyloses from starch solution is separated in form of crystal. This is the crystallisation phenomenon of starch molecule. The various chemical compounds indicted responsible for starch crystallisation are known as crystallising agents.

Lecture 8

Glycogen: definition, properties, Cellulose, pectin, gums: Occurrences, properties, uses; **Physical properties of carbohydrates.**

1.3.11. Glycogen:

Glycogen is a multibranched polysaccharide of glucose that serves as a form of energy storage in humans, animals, and fungi. The polysaccharide structure represents the main storage form of glucose in the body.

In humans, glycogen is made and stored primarily in the cells of the liver and the muscles, hydrated with three or four parts of water. Glycogen functions as the secondary long-term energy storage, with the primary energy stores being fats held in adipose tissue. Muscle glycogen is converted into glucose by muscle cells, and liver glycogen converts to glucose for use throughout the body including the central nervous system.

Molecular weight: $1-2 \times 10^7$

Structure: Glycogen is a branched biopolymer consisting of linear chain of glucose residues with further chains branching off every 8 to 12 glucose or so. Glucoses are linked together linearly by α -1:4 glycosidic bonds from one glucose to the next. Branches are linked to the chains from which they are branching off by α -1:6 glycosidic bonds between the first glucose of the new branch and glucose on the stem chain.

1.3.12. Cellulose and Hemicellulose:

Cellulose and hemicellulose are two types of natural polymers that are mainly found in the plant cell walls and are important components of natural lignocellulosic materials. But, these two

components are different in the chemical composition and the structure. The key difference between cellulose and hemicellulose is that cellulose is an organic polysaccharide molecule whereas hemicellulose is a matrix of polysaccharides.

Cellulose:

Cellulose is an organic polysaccharide molecule with the molecular formula $(C_6H_{10}O_5)_n$. It has a linear chain of several hundred to thousands of D-glucose units. Cellulose is a natural polymeric compound found in many natural materials; for instance, it is the structural component of the primary cell wall in green plants. It can be also found in many forms of algae species. Cellulose is the commonest organic polymer on Earth. Many natural compounds are rich in cellulose; for example, the cellulose content of wood, cotton fiber, and dried hemp are 40–50%, 90%, and 57% respectively.

Structure: Cellulose is an un-branched polymeric molecule and has 7,000–15,000 glucose molecules per polymer.

Hemicellulose:

Hemicellulose, also known as polyose, is a matrix of polysaccharides, such as arabinoxylans, that exist along with cellulose in almost all the plant cell walls. It is a polysaccharide that is present in the biomass of most plants; about 20%-30% dry weight of plants. Hemicellulose, combined with cellulose, provides physical and structural strength to the cell wall. In addition to glucose, the other structural components in hemicelluloses are xylose, galactose, mannose, rhamnose, and arabinose. Hemicellulose has shorter chains of 500 and 3000 sugar units with a branched structure.

Structure: Hemicellulose contains shorter chains of 500–3,000 sugar units and it is a branched polymer.

1.3.13. Pectin and Its Derivatives:

Pectin and its derivatives have the following characteristics:

- Pectin are made up of chains of repeating units like sugar acids.
- Pectins are common in fruits and vegetables and are gum like (They are found in and between cell walls and help hold the plant cells together).
- Pectins are soluble in water, especially in hot water.
- Pectins in colloidal solution contribute viscosity to tomato paste and stabilize the fine particles in orange juice from settling out.
- Pectins in solution form gels when sugar and acid are added and this is basis of jelly preparation.

1.3.14.Gums:

Gums are mainly classified into four categories:

1. Seed gums: Gum guar, Locust ban gum
2. Plant exudate gums: Gum arabic or gum acacia
3. Seaweed extracts: Agar, algin, carragunan
4. Microbial gum: Dextran, mannan etc.

Gum Arabic or gum acacia:

This gum is extracted from acacia tree. Molecular weight of gum arabic is 1000-1200. It is a polymer of galactose, arabinose, ramnose and gluconic acid. It a branched molecule whose backbone is galactose unit. The molecules are linked together in this branched structure by alpha 1:3, alpha 1:4 and alpha 1:6 glycosidic linkages.

Viscosity of gum arabic solution is low in comparison to other gums. With increasing concentration, above 40%, the viscosity and adhesive properties of gum arabic increased.

Industrial uses:

1. It is used in confectionary industries as emulsifier.
2. It is used in verities of food and pharmaceutical industries as thickening and emulsifying agents.
3. It is used as clarifying agent in wine and beer industries.
4. It is used as foam stabilizer.
5. It is used as a moisture retaining agent in baked items.

Agar:

Agar is extracted from red seaweeds. Agar is a polysaccharide. It is a calcium salt of strongly ionized half ester of galactan substance. The linkages in agar molecules are alpha 1:3 and alpha 1:4 glycosidic linkages.

Industrial uses:

1. It acts as gelatinizing agent in various food and pharmaceutical industries.
2. It acts as a stabilizing agent in bakery products and in confectionary.
3. It is also used as solidifying agent in media preparation at microbiology labs.

Algin:

It is extracted from brown seaweeds. It is sodium salt or ester of polymannuronic acid.

Industrial uses:

1. It is used as stabilizing agent in preparation of ice-cream.
2. It is used as texture improving agent in preparation of cheese.
3. It is used as emulsifying agent in different types of syrup preparations.

Lecture 1

Protein: Definition, Classification and Structure

2.1 Definition: The term “**protein**” derives from the Greek word “*proteios*”, that means primary and was suggested for the first time by Jöns Jacob Berzelius, one of the fathers of modern chemistry. Proteins are large biomolecules or macromolecules composed of one or more long chains of amino acids and are an essential part of all living organisms, especially as structural components of body tissues such as muscle, hair, etc., and as enzymes and antibodies.

2.2 Classification of Protein:

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) Histories
- e) Protamine
- f) prolamines
- g) Scleroproteins

ii) Conjugated 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

2.3 Structure of Protein:

Protein structure is the three-dimensional arrangement of atoms in a protein molecule. Proteins are polymers — specifically polypeptides — formed from sequences of amino acids, the monomers of the polymer. To be able to perform their biological function, proteins fold into one or more specific spatial conformations driven by a number of non-covalent interactions such as hydrogen bonding, ionic interactions, Vander Waals forces, and hydrophobic packing. To understand the functions of proteins at a molecular level, it is often necessary to determine their three-dimensional structure which employs techniques such as X-ray crystallography, NMR spectroscopy, and dual polarisation interferometry.

Primary structure

The primary structure of a protein refers to the linear sequence of amino acids in the polypeptide chain. The primary structure is held together by covalent bonds such as peptide bonds, which are made during the process of protein biosynthesis. The two ends of the polypeptide chain are referred to as the carboxyl terminus (C-terminus) and the amino terminus (N-terminus) based on the nature of the free group on each extremity. Counting of residues always starts at the N-terminal end (NH₂-group), which is the end where the amino group is not involved in a peptide bond.

Secondary structure

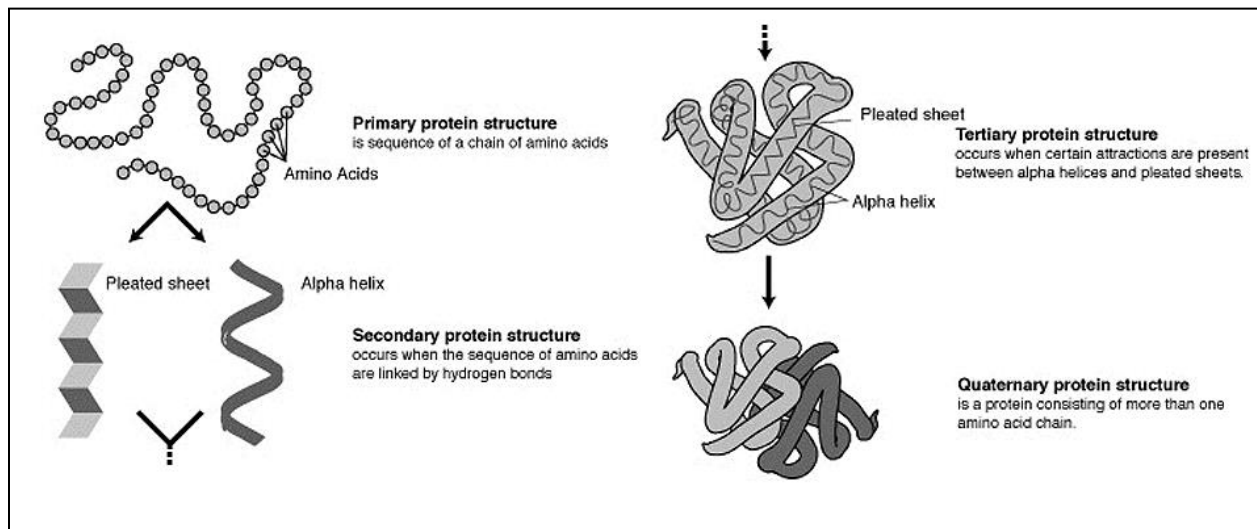
Secondary structure refers to highly regular local sub-structures on the actual polypeptide backbone chain. Two main types of secondary structure, the α -helix and the β -sheets, were suggested in 1951 by Linus Pauling. These secondary structures are defined by patterns of hydrogen bonds between the main-chain peptide groups.

Tertiary structure

Tertiary structure refers to the three-dimensional structure of monomeric and multimeric protein molecules. The α -helices and β -pleated-sheets are folded into a compact globular structure. The folding is driven by the non-specific hydrophobic interactions, the burial of hydrophobic residues from water, but the structure is stable only when the parts of a protein domain are locked into place by specific tertiary interactions, such as salt bridges, hydrogen bonds, and the tight packing of side chains and disulfide bonds.

Quaternary structure

Quaternary structure is the three-dimensional structure of a multi-subunit protein and is stabilized by the non-covalent interactions and disulfide bonds. Complexes of two or more polypeptides (i.e. multiple subunits) are called multimers. Specifically it would be called a dimer if it contains two subunits, a trimer if it contains three subunits, a tetramer if it contains four subunits, and a pentamer if it contains five subunits. The subunits are frequently related to one another by symmetry operations, such as a 2-fold axis in a dimer. Multimers made up of identical subunits are referred to with a prefix of "homo-" (e.g. a homotetramer) and those made up of different subunits are referred to with a prefix of "hetero-", for example, a heterotetramer, such as the two alpha and two beta chains of hemoglobin.



Lecture 2

Properties of proteins: Amphoterism, hydration, binding of ions, precipitation with antibodies, gel formation of proteins

2.4 Properties of Proteins:

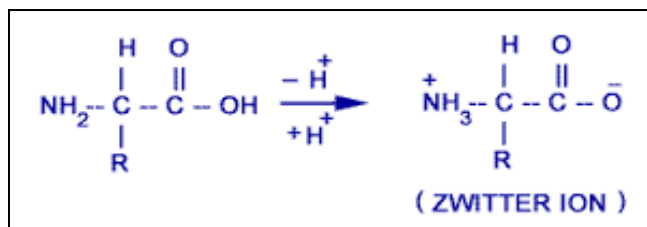
- i) Optical Property
- ii) Colloidal
- iii) Solubility
- iv) Amphoteric Nature
- v) Denaturation
- vi) Hydration
- vii) Precipitation
- viii) Gel formation

2.5 Function of Protein:

- 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

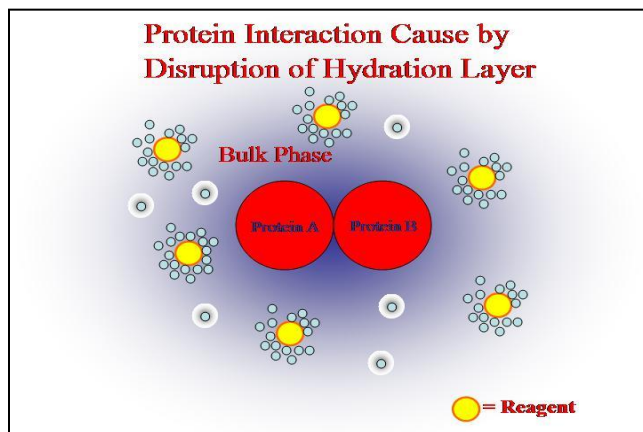
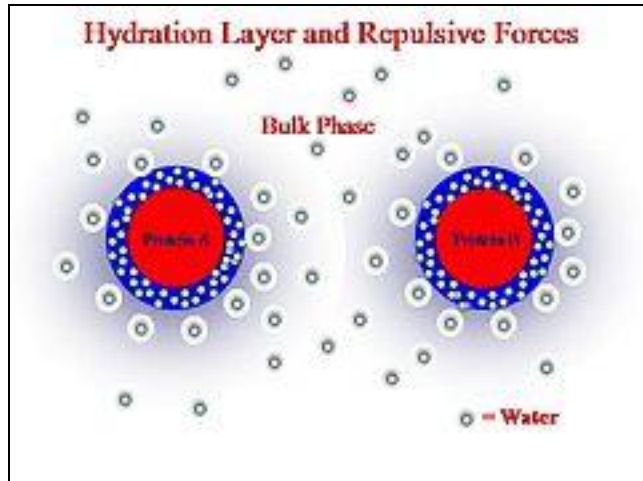
2.6 Amphoteric nature of Protein:

Amino acids due to the presence of their ionizable α -amino and α -carboxylic group can act sometimes as acids and some time as bases depending on the pH of their media.



2.7 Hydration Layer Formation:

A solvation shell is the solvent interface of any chemical compound or biomolecule that constitutes the solute. When the solvent is water it is often referred to as a hydration shell or hydration sphere. The hydration shell (also sometimes called hydration layer) that forms around proteins is of particular importance in biochemistry. This interaction of the protein surface with the surrounding water is often referred to as protein hydration and is fundamental to the activity of the protein. The hydration layer around a protein has been found to have dynamics distinct from the bulk water to a distance of 1 nm. The duration of contact of a specific water molecule with the protein surface may be in the subnanosecond range.



2.8 Binding of ions, precipitation with antibodies, gel formation of proteins

Binding of ions: The charge on the protein affects its behavior in ion exchange. Proteins contain many ionizable groups on the side chains of their amino acids including their amino - and carboxyl - termini. These include basic groups on the side chains of lysine, arginine and histidine and acidic groups on the side chains of glutamate, aspartate, cysteine and tyrosine. The pH of the solution, the pK of the side chain and the side chain's environment influence the charge on each side chain. In general terms, as the pH of a solution increases, deprotonation of the acidic and basic groups on proteins occur, so that carboxyl groups are converted to carboxylate anions (R-COOH to R-COO⁻) and ammonium groups are converted to amino groups (R-NH₃⁺ to R-NH₂). In proteins the isoelectric point (pI) is defined as the pH at which a protein has no net charge. When the pH > pI, a protein has a net negative charge and when the pH < pI, a protein has a net positive charge. The pI varies from protein to protein.

Precipitation with antibodies: Antibodies are protein components of an adaptive immune system whose main function is to bind antigens, or foreign substances in the body, and target them for destruction. Antibodies can be secreted into the extracellular environment or anchored in the membranes of specialized B cells known as plasma cells.

Gel formation: Gel is an intermediary form between solid and liquid. It is the cross-linking among polymeric molecules which make an intermolecular network within a liquid medium. In food systems, this liquid is water, a solvent which affects the nature and the magnitude of intermolecular strengths that keep the integrity of the polymeric network. This network retains water, avoiding losses. Gels can be described by their capacity to immobilize liquids, by their macromolecular structure, by their texture, and by their rheological properties. Gel formed from protein was primarily described as a two-step process:

1. The first step involves changes in the conformation (usually induced by heat) or partial denaturation of the protein molecule. With denaturation, the dispersion velocity increases as a result of increasing molecular dimensions caused by unfolding of the protein molecule. In this protein dispersion, the first features of an elastic solid appear.
2. In the second step, a gradual association or molecule aggregations of denatured proteins leads to an exponential increase of viscosity, and to the formation of a continuous network. Process formation in this step is slower, in comparison to the first step, and ends when an organized network is formed.

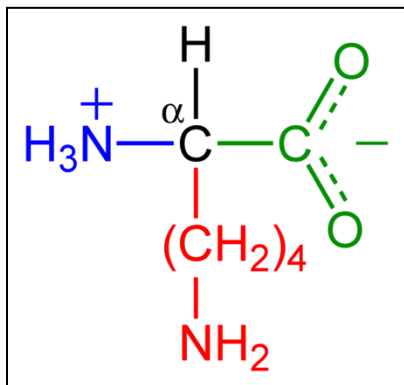
The establishment of gel networks at 85 to 90°C is attributed to the formation of covalent linkages, to the changes of thiol group to disulphide linkages, and to hydrophobic interactions. These interactions between nonpolar segments of adjacent polypeptides occur only if these polypeptides are opened, induced by heating. Cooling increases the hydrogen bonds. The change reaction from thiol group to disulphide linkage is important for thermal gelation, given that it produces crossed covalent linkages involving and stabilizing the gel matrix. The elasticity property of gel is directly proportional to the density of cross-linking in the network.

The nature and properties of gels are influenced by several factors, such as protein concentration, solution pH, nature, and concentration of the electrolyte. Gelation can occur during heating or cooling and depends on the nature of the protein and on the process itself. The heat-induced formation of translucent gel network involves the ordinate association of unfolded chains of polypeptides through non-covalent bonds (e.g. hydrogen bounds, ionic and hydrophobic interactions) and in some cases, through covalent bonds (disulphide linkages).

The protein-protein, non-covalent interactions occur during the formation of reversible and non-reversible gels. The crossed intermolecular linkages between developed chains of polypeptides vary extensively and are essential to gel formation. The kind and the extension of non-covalent interactions, such as hydrophobic and 'Van der Waals' interactions, hydrogen bonds and ionic interactions, are related to the nature of the protein, to its concentration, to the solution pH, to the denaturation intensity caused by heating and by the ionic medium, and interfere with the attractive and repulsive strengths of the three-dimensional network. Therefore, excess attractive strength cause coagulation, and excess repulsive strength causes dissolution of the network's structure.

2.9 Zwitterions

An amino acid is in a zwitterionic state when the carboxylic acid group is deprotonated and the amino group is protonated, simultaneously. Zwitterions are dipole ions—meaning that these molecules have two charges, both a positive and a negative charge. The pH of the water solution is a factor determining the state of protonation. Such a state leaves the carboxylic end negatively charged ($-\text{COO}^-$) and the adjacent amino end positively charged ($-\text{NH}_3^+$). The carboxyl group ($-\text{COO}^-$) is deprotonated first because the pKa is about 2 and the pKa of the amine group ($-\text{NH}_3^+$) is about 9. The net charge for the protein in zwitterionic form is zero. ^[1] Molecules which behave in this fashion are called amphoteric.



Lecture 3

Amino acids: Essential and non essential amino acids, their structures, deficiency disease; Acidic and basic amino acids

2.10 Amino Acid Classification

Non-polar Amino Acids

Aliphatic : glycine, alanine, valine, isoleucine, leucine

Aromatic : phenylalanine, tryptophan.

Cyclic : Proline

Polar Amino Acids

Sulfur-Containing : cysteine, methionine

Hydroxyl-Containing : serine, threonine

Aromatic : tyrosine

Acidic Amide : asparagine, glutamine

Charged Amino Acids (at physiological pH)

Acidic : aspartic acid, glutamic acid

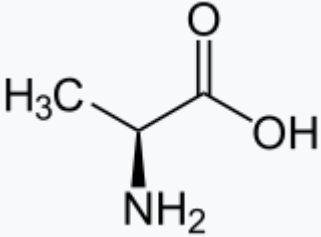
Basic : histidine, lysine, arginine

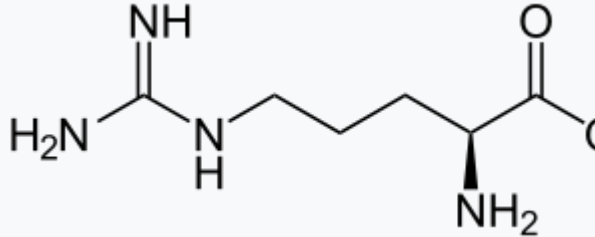
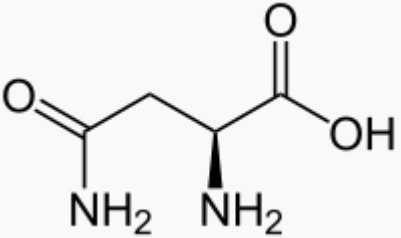
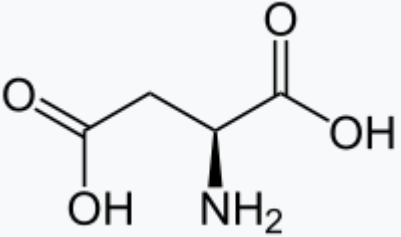
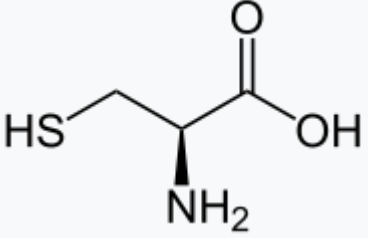
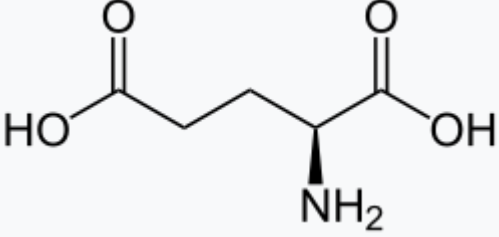
2.11 Deficiency disease:

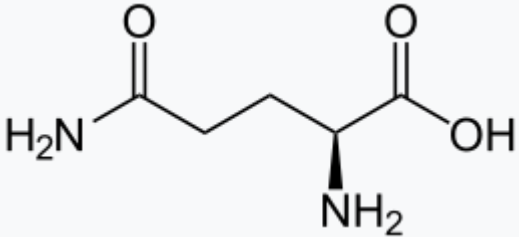
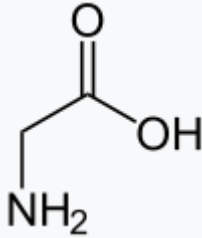
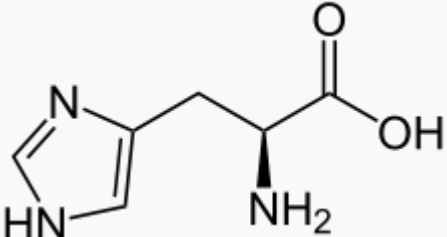
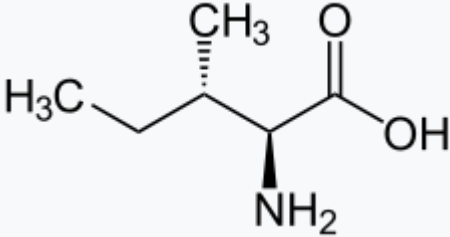
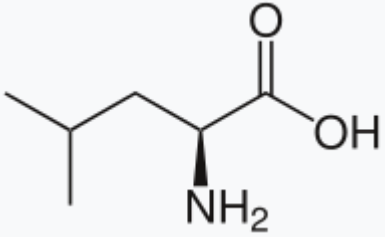
Kwashiorkor is a severe form of malnutrition, caused by a deficiency in dietary protein mainly sulfur containing amino acids (methionine, cysteine, glutathione), characterized by edema, irritability, ulcerating dermatoses, and an enlarged liver with fatty infiltrates. The extreme lack of protein causes an osmotic imbalance in the gastro-intestinal system causing swelling of the gut diagnosed as an edema or retention of water.

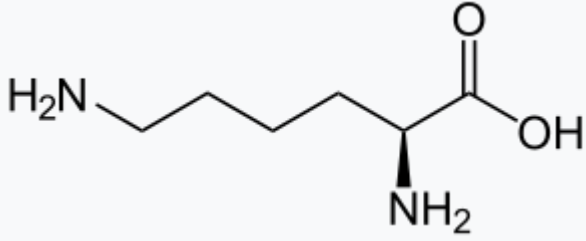
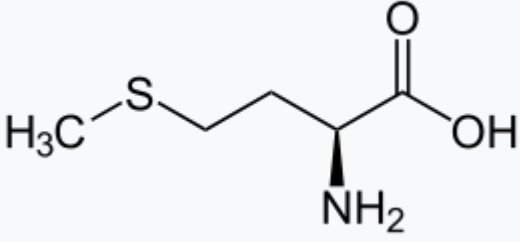
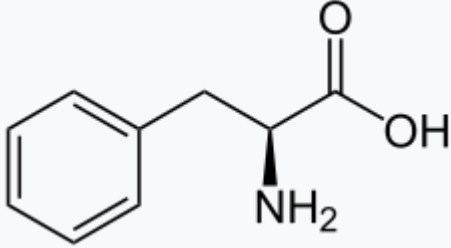
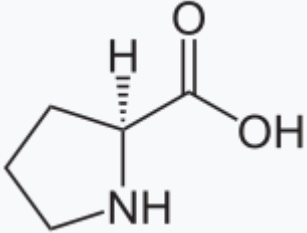
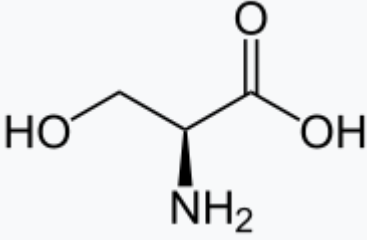


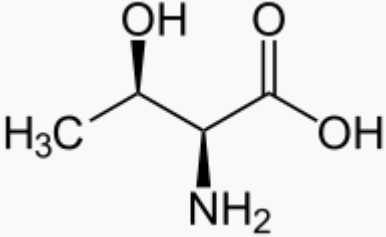
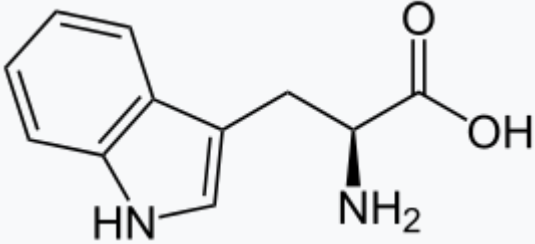
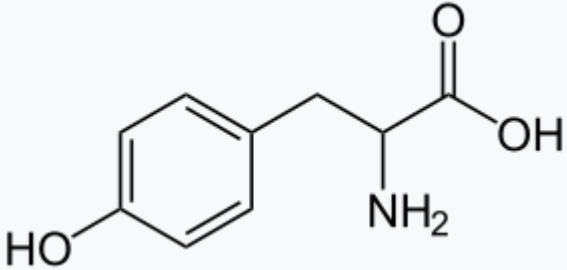
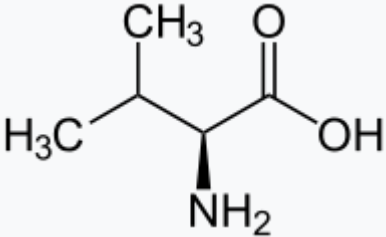
2.12 Essential and non essential amino acids and their structures

Amino Acid	3-Letter Abbreviation	1-Letter Abbreviation	Class of Amino Acid (Side Chain)	Structure
Alanine	Ala	A	Aliphatic, nonpolar	 <chem>C[C@@H](N)C(=O)O</chem>

Amino Acid	3-Letter Abbreviation	1-Letter Abbreviation	Class of Amino Acid (Side Chain)	Structure
Arginine	Arg	R	Basic	
Asparagine	Asn	N	Acidic, polar	
Aspartate	Asp	D	Acidic	
Cysteine	Cys	C	Hydroxyl or Sulfur-Containing, polar	
Glutamate	Glu	E	Acidic	

Amino Acid	3-Letter Abbreviation	1-Letter Abbreviation	Class of Amino Acid (Side Chain)	Structure
Glutamine	Gln	Q	Acidic, polar	
Glycine	Gly	G	Aliphatic, nonpolar	
Histidine	His	H	Basic	
Isoleucine	Ile	I	Aliphatic, nonpolar	
Leucine	Leu	L	Aliphatic, nonpolar	

Amino Acid	3-Letter Abbreviation	1-Letter Abbreviation	Class of Amino Acid (Side Chain)	Structure
Lysine	Lys	K	Basic	
Methionine	Met	M	Hydroxyl or Sulfur-Containing, nonpolar	
Phenylalanine	Phe	F	Aromatic	
Proline	Pro	P	Cyclic	
Serine	Ser	S	Hydroxyl or Sulfur-Containing, polar	

Amino Acid	3-Letter Abbreviation	1-Letter Abbreviation	Class of Amino Acid (Side Chain)	Structure
Threonine	Thr	T	Hydroxyl or Sulfur-Containing, polar	
Tryptophan	Trp	W	Aromatic	
Tyrosine	Tyr	Y	Aromatic	
Valine	Val	V	Aliphatic, nonpolar	

Lecture 4

Purification of proteins: Different processes; Electrophoresis of protein: definition, process, applications

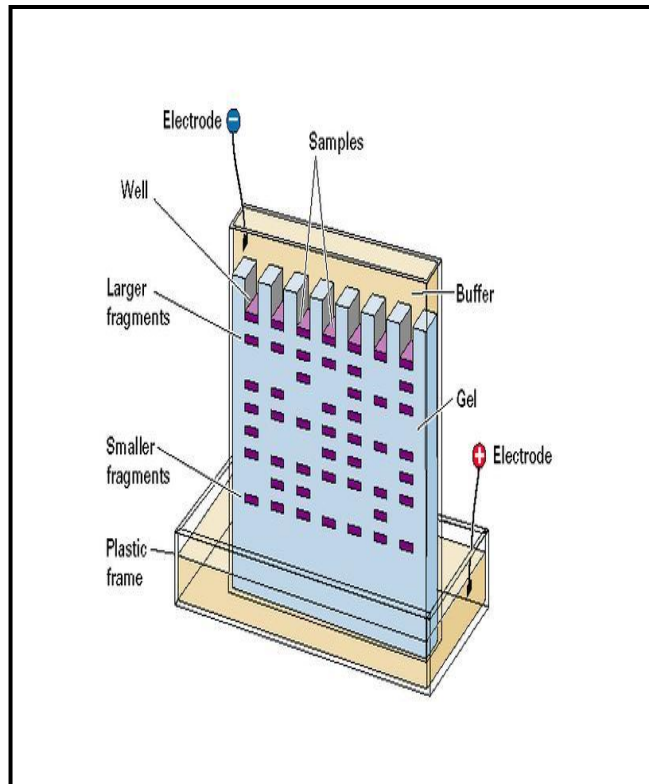
2.13 Definition:

Electrophoresis is a technique used to separate and sometimes purify macromolecules - especially proteins and nucleic acids - that differ in size, charge or conformation. It is one of the most widely-used techniques in biochemistry and molecular biology.

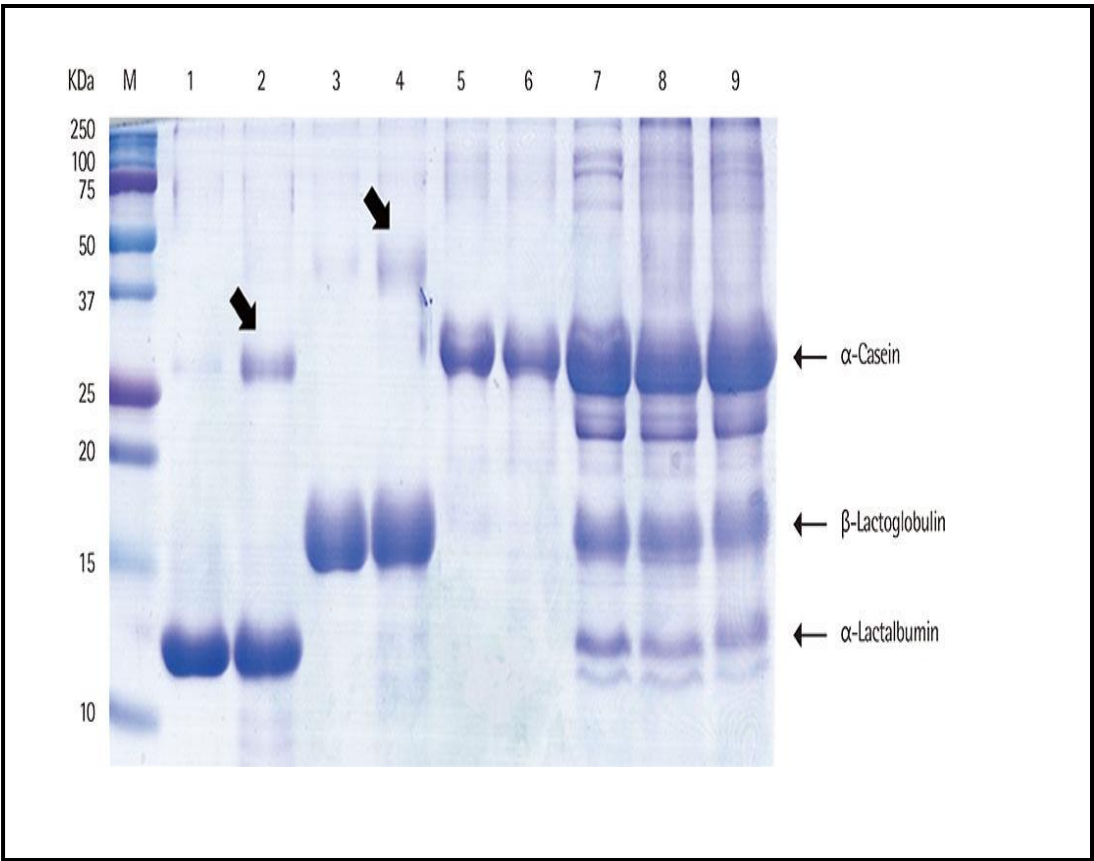
2.14 Principle

The principle of electrophoresis states that in the presence of an electric field, a charged particle moves toward the region of an opposite charge. When the particle has unequal charge distribution in its chemical bonds, it aligns on the electric potential.

In the fields of molecular biology and biochemistry, electrophoresis is a useful analytical technique where macromolecules of varying sizes and densities are separated. Complex proteins and nucleic acids that undergo electrophoresis move through a gel matrix that is primarily composed of polymerized agarose or polyacrylamide. Agarose is a polysaccharide that forms a gelatin-like substance when dissolved in boiling water. Polyacrylamide is a type of solid gel created by polymerization of acrylamide solutions through the addition of ammonium persulfate coupled with tetramethylenediamine.



When a negatively charged particle travels along an electric field, it tends to migrate toward the anode and move against frictional force. The bigger the particle, the slower it moves.



2.15 Applications

Electrophoresis is a molecular separation technique that involves the use of high voltage electric current for inducing the movement of charged molecules like proteins, DNA, nucleic acids in a support medium. The movement of charged molecules is called mobility.

- Protein Analysis
- DNA Analysis
- Antibiotics Analysis
- Vaccine Analysis

Lecture 5

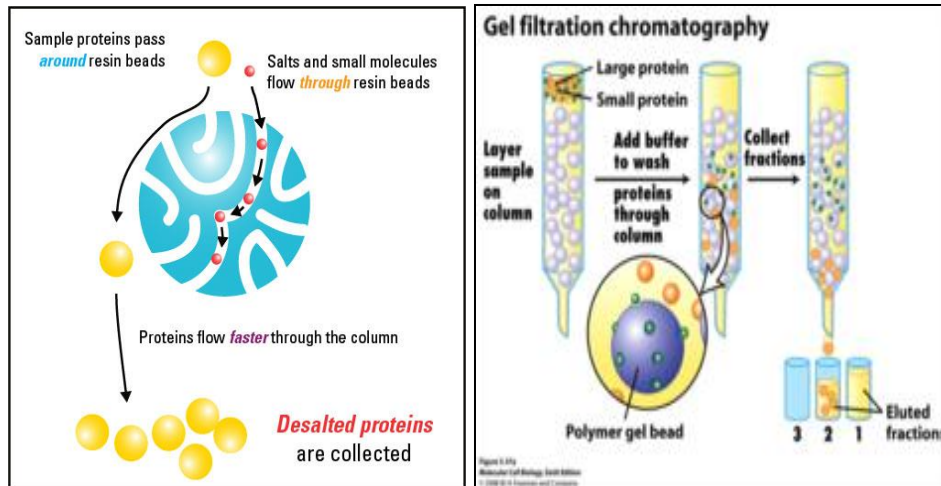
Gel filtration: definition, process, applications; Spectrophotometric analysis of protein, Lambert-Beer's law

2.16 Gel filtration:

Definition: Gel filtration chromatography is a separation based on size. It is also called molecular exclusion or gel permeation chromatography. In gel filtration chromatography, the stationary phase consists of porous beads with a well-defined range of pore sizes.

2.17 Principle

Gel filtration is well suited for biomolecules that may be sensitive to changes in pH, concentration of metal ions or co-factors and harsh environmental conditions. Separations can be performed in the presence of essential ions or cofactors, detergents, urea, guanidine hydrochloride, at high or low ionic strength, at 37°C or in the cold room according to the requirements of the experiment. Purified proteins can be collected in any chosen buffer. Gel filtration can be used directly after ion exchange, chromatofocusing, hydrophobic interaction, or affinity, since the buffer composition will not generally affect the final separation.



2.18 Application

The main application of gel-filtration chromatography is the fractionation of proteins and other water-soluble polymers.

2.19 Spectrophotometric analysis of protein

Definition: Spectrophotometry is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength.

2.20 Principle:

Lambert-Beer's law

Lambert's law stated that absorbance of a material sample is directly proportional to its thickness (path length). Much later, August Beer discovered another attenuation relation in 1852. Beer's law stated that absorbance is proportional to the concentrations of the attenuating species in the material sample.

Lambert's Law is expressed as:

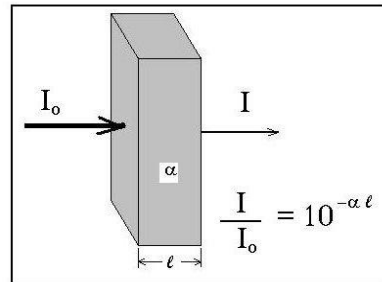
$$\text{Transmittance (T)} = \frac{\text{Intensity of transmitted light (I)}}{\text{Intensity of incident light (I}_0)}$$

where transmittance is the ratio of the amount of light transmitted to the amount of light that initially fell on the surface.

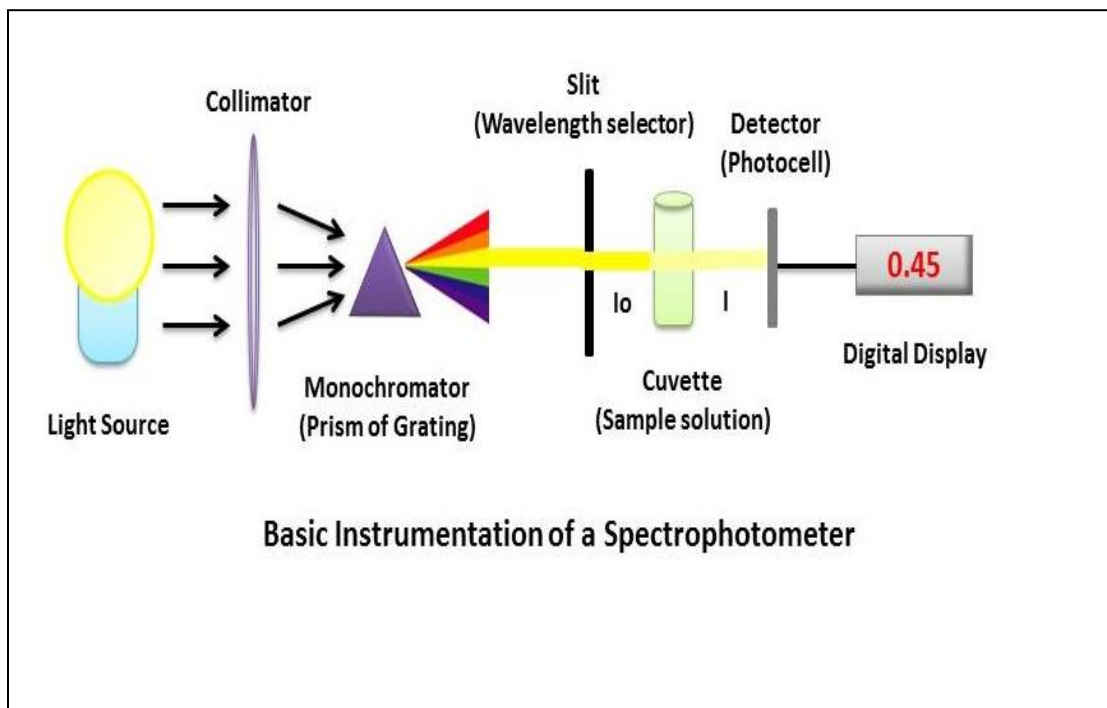
On the other hand, Beer's Law is expressed as:

$$\text{Absorbance (A)} = \text{Extinction coefficient or Molar absorptivity (e)} \times \text{Concentration (C)} \times \text{Path length (L)}$$

where absorbance is the negative logarithm of transmittance.



The Beer Lambert Law

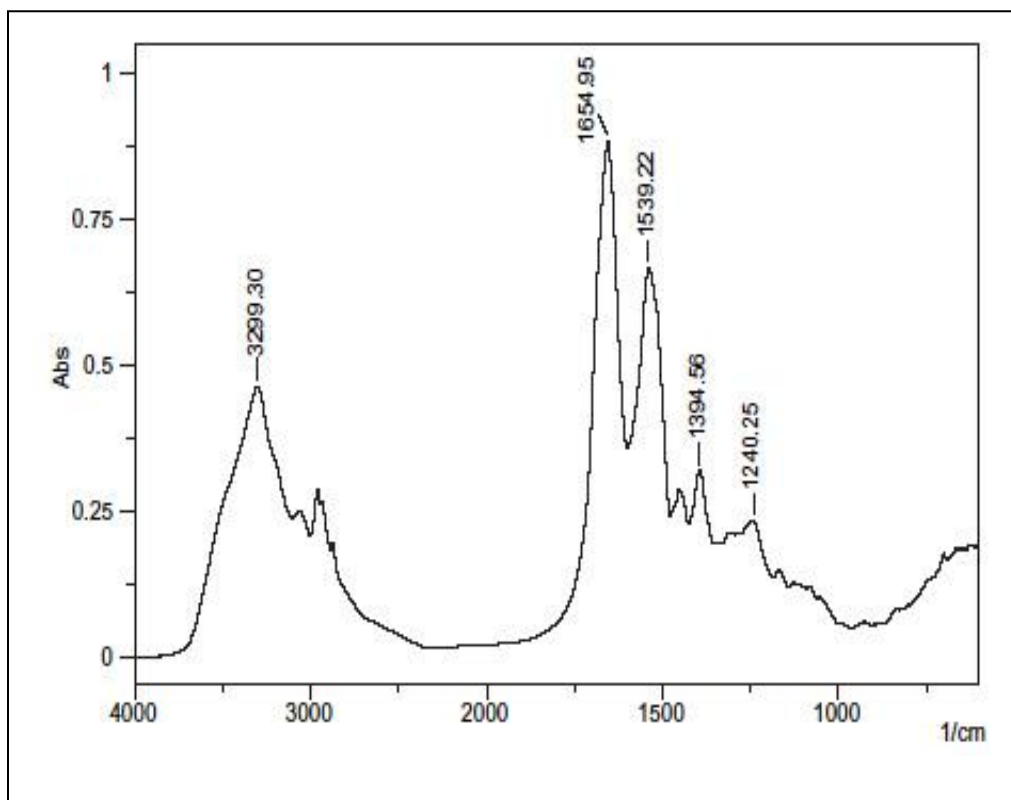


Basic Instrumentation of a Spectrophotometer

2.21 Analysis of Protein:

Principle: Basically, spectrophotometry is one of the most widely used analytical procedures in biochemistry. It is commonly used to estimate the level of an analyte in solution and is ideal for simple routine determination of small quantities of materials. This method is based on the two laws of light absorption by solutions, namely Lambert's Law and Beer's Law. Since

proteins absorb light at a specific wavelength, a spectrophotometer can be used to directly measure the concentration of a purified protein in solution.



Lecture 6

Paper chromatography, Thin layer Chromatography: Definition, processes, applications

2.22 Paper chromatography

Definition:

It is an analytical method used to separate colored chemicals or substances. It is primarily used as a teaching tool, having been replaced by other chromatography methods, such as thin-layer chromatography. A paper chromatography variant, two-dimensional chromatography involves using two solvents and rotating the paper 90° in between.

R_f value: The retention factor (R_f) may be defined as the ratio of the distance traveled by the substance to the distance traveled by the solvent. R_f values are usually expressed as a fraction of two decimal places. If R_f value of a solution is zero, the solute remains in the stationary phase and thus it is immobile. If R_f value = 1 then the solute has no affinity for the stationary phase and travels with the solvent front.

2.23 Principle:

This is useful for separating complex mixtures of compounds having similar polarity, for example, amino acids. The setup has three components. The mobile phase is a solution that travels up the stationary phase, due to capillary action. The mobile phase is generally an alcohol solvent mixture, while the stationary phase is a strip of chromatography paper, also called a chromatogram. A chromatographic method is called adsorption chromatography if the stationary phase is solid. Paper chromatography is one method for testing the purity of compounds and identifying substances. Paper chromatography is a useful technique because it is relatively quick and requires only small quantities of material. Separations in paper chromatography involve the same principles as those in thin layer chromatography, as it is a type of thin layer chromatography. In paper chromatography, substances are distributed between a stationary phase and a mobile phase. The stationary phase is the water trapped between the cellulose fibers of the paper. The mobile phase is a developing solution that travels up the stationary phase, carrying the samples with it. Components of the sample will separate readily according to how strongly they adsorb onto the stationary phase versus how readily they dissolve in the mobile phase.

When a colored chemical sample is placed on a filter paper, the colors separate from the sample by placing one end of the paper in a solvent. The solvent diffuses up the paper, dissolving the various molecules in the sample according to the polarities of the molecules and the solvent. If the sample contains more than one color, that means it must have more than one kind of molecule. Because of the different chemical structures of each kind of molecule, the chances are very high that each molecule will have at least a slightly different polarity, giving each molecule a different solubility in the solvent. The unequal solubility causes the various color molecules to leave solution at different places as the solvent continues to move up the paper. The more soluble a molecule is, the higher it will migrate up the paper. If a chemical is very non-polar it will not dissolve at all in a very polar solvent. This is the same for a very polar chemical and a very non-polar solvent. It is very important to note that when using water (a very polar substance) as a solvent, the more polar the color, the higher it will rise on the paper.

2.24 Application:

- a) In the separation, purification, and identification of a great range of compounds.
- b) In forensic studies paper chromatography is used in crime scene investigation and DNA and RNA sequencing along with other studies.
- c) Analytical chemistry technique for identifying and separating colored mixtures like pigments.
- d) Sugars, amino acids, lipids and nucleic acids and other biomolecules can be easily identified.

2.25 Types of Paper Chromatography:

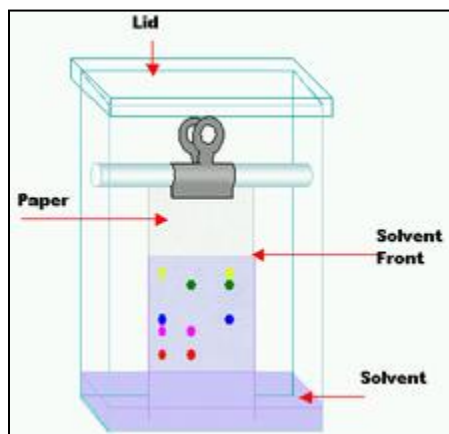
1. Descending Paper Chromatography-In this type, development of the chromatogram is done by allowing the solvent to travel down the paper. Here, mobile phase is placed in solvent holder at the top. The spot is kept at the top and above solvent flow down the paper from above.

2. Ascending Paper Chromatography-Here the solvent travels up the chromatographic paper. Both Descending and Ascending Paper Chromatography are used for the separation of organic and inorganic substances.

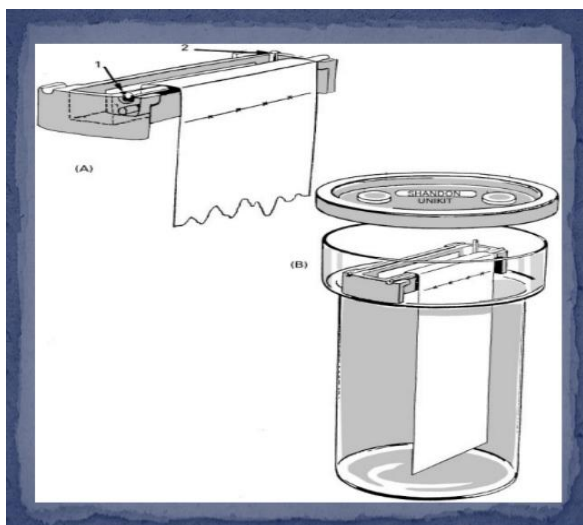
3. Ascending-Descending Paper Chromatography-It is the hybrid of both of the above techniques. The upper part of Ascending Chromatography can be folded over a rod in order to allow the paper to become Descending after crossing the rod.

4. Radial Paper Chromatography-It is also called Circular Chromatography. Here a circular filter paper is taken and the sample is deposited at the center of the paper. After drying the spot, the filter paper is tied horizontally on a Petri dish containing solvent, so that the wick of the paper is dipped in the solvent. The solvent rises through the wick and the components are separated into concentric circles.

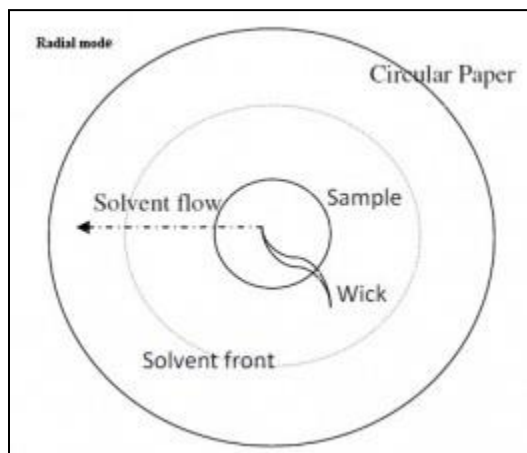
5. Two-Dimensional Paper Chromatography-In this technique a square or rectangular paper is used. Here the sample is applied to one of the corners and development is performed at right angle to the direction of the first run.



Ascending Paper Chromatography



Descending Paper Chromatography



Radial Paper Chromatograph

2.26 TLC or Thin Layer Chromatography

TLC is a type of planar chromatography.

- It is routinely used by researchers in the field of phyto-chemicals, biochemistry, and so forth, to identify the components in a compound mixture, like alkaloids, phospholipids, and amino acids.
- It is a semi quantitative method consisting of analysis.
- High performance thin layer chromatography (HPTLC) is the more sophisticated or more precise quantitative version.

2.27 Principle

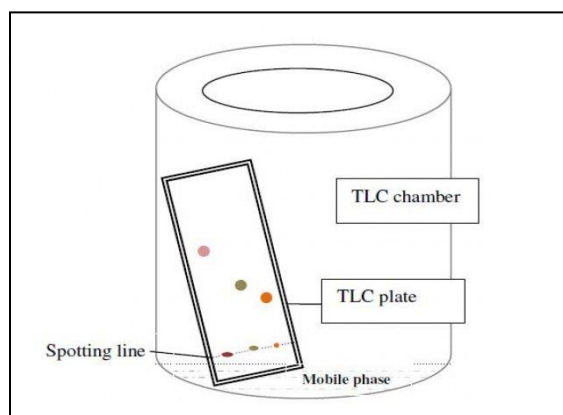
Similar to other chromatographic methods, thin layer chromatography is also based on the principle of separation.

1. The separation depends on the relative affinity of compounds towards stationary and the mobile phase.
2. The compounds under the influence of the mobile phase (driven by capillary action) travel over the surface of the stationary phase. During this movement, the compounds with higher affinity to stationary phase travel slowly while the others travel faster. Thus, separation of components in the mixture is achieved.
3. Once separation occurs, the individual components are visualized as spots at a respective level of travel on the plate. Their nature or character are identified by means of suitable detection techniques.

2.28 System Components

TLC system components consists of

1. **TLC plates**, preferably readymade with a stationary phase: These are stable and chemically inert plates, where a thin layer of stationary phase is applied on its whole surface layer. The stationary phase on the plates is of uniform thickness and is in a fine particle size.
2. **TLC chamber**. This is used for the development of TLC plate. The chamber maintains a uniform environment inside for proper development of spots. It also prevents the evaporation of solvents, and keeps the process dust free.
3. **Mobile phase**. This comprises of a solvent or solvent mixture The mobile phase used should be particulate-free and of the highest purity for proper development of TLC spots. The solvents recommended are chemically inert with the sample, a stationary phase.



2.29 Advantages

- It is a simple process with a short development time.
- It helps with the visualization of separated compound spots easily.
- The method helps to identify the individual compounds.
- It helps in isolating of most of the compounds.

- The separation process is faster and the selectivity for compounds is higher (even small differences in chemistry is enough for clear separation).
- The purity standards of the given sample can be assessed easily.
- It is a cheaper chromatographic technique.

2.30 Applications

1. To check the purity of given samples.
2. Identification of compounds like acids, alcohols, proteins, alkaloids, amines, antibiotics, and more.
3. To evaluate the reaction process by assessment of intermediates, reaction course, and so forth.
4. To purify samples, i.e for the purification process.
5. To keep a check on the performance of other separation processes.

Being a semi quantitative technique, TLC is used more for rapid qualitative measurements than for quantitative purposes. But due its rapidity of results, easy handling and inexpensive procedure, it finds its application as one of the most widely used chromatography techniques.

Lecture 7

Overview of GLC, HPLC, Ion exchange chromatography, Gas chromatography

2.31 Gas Chromatography:

Definition: It is a technique where by the components of a mixture (sample) in the gaseous state are separated as the sample passes over a stationary liquid or solid phase and a gaseous mobile phase. Based on stationary phase G.C classified into two types. Gas Solid Chromatography (G.S.C) and Gas Liquid Chromatography (G.L.C)

2.32 Gas Liquid Chromatography

In gas-liquid chromatography the mobile phase is an unreactive gas, such as nitrogen (the carrier gas), and the stationary phase comprises of a small amount of non volatile liquid held on a finely-divided inert solid support.

Principle: Gas liquid chromatography runs on the principle of partition. In GLC the components of vaporize samples are fractionated due to partition between a gaseous mobile phase and a liquid stationary phase held in column.

2.33 HPLC (High Performance Liquid Chromatography)

Definition: HPLC is a form of liquid chromatography used to separate compounds that are dissolved in solution. It is characterized by the use of high pressure to push a mobile phase solution through a column of stationary phase allowing separation of complex mixtures with high resolution. Mobile phase is Liquid and Stationary phase is Solid or Liquid.

Principle: The process involves the interaction of the compounds in the analyte or sample across an immobile surface (stationary phase). The compounds bind at specific regions of stationary phase based on certain physical and chemical properties. These bound molecules are then eluted with a suitable buffer and the same are collected with time. The properties are Polarity, Charge, Molecular weight, functional group.

2.34 Ion-exchange chromatography: Here the stationary bed has an ionically charged surface of opposite charge to the sample ions. This technique is used almost exclusively with ionic or ionizable samples. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

2.35 Size exclusion chromatography: Here the column is filled with material having precisely controlled pore sizes, and the sample is simply screened or filtered according to its solvated molecular size. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later. This technique is also called gel filtration or gel permeation chromatography.

2.36 Adsorption chromatography: Here stationary phase is an adsorbent (like silica gel) and the separation is based on repeated adsorption-desorption steps. Concerning the Adsorption chromatography two modes are defined depending on the relative polarity of the two phases: Normal-phase chromatography; the stationary bed is strongly polar in nature (e.g., silica gel) and the mobile phase is nonpolar (such as n-hexane or tetrahydrofuran). Polar samples are thus retained on the polar surface of the column packing longer than less polar materials. Reversed-phase chromatography; the stationary bed is nonpolar (hydrophobic) in nature. The mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile. Here the more nonpolar the material is, the longer it will be retained.

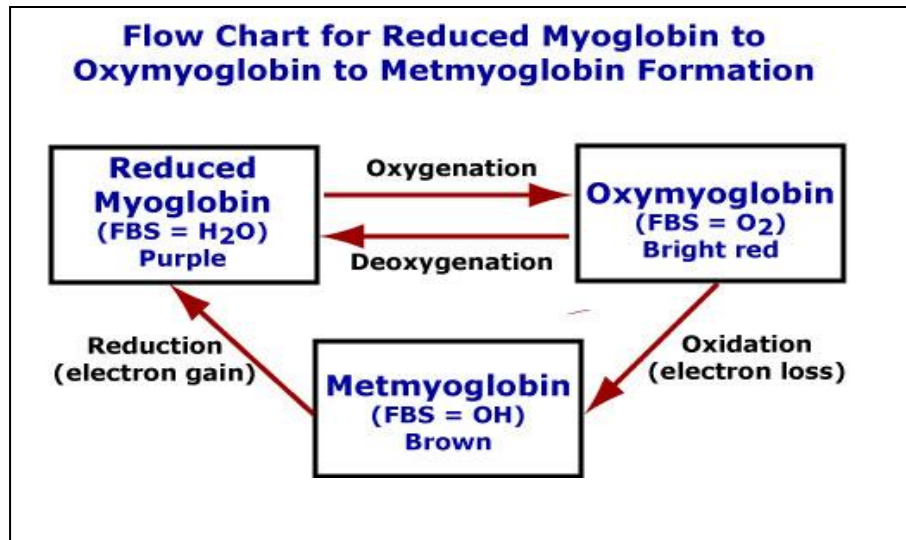
Lecture 8

Different types of food proteins: Meat protein: Myoglobin, changes in colour during curing of meat, Egg proteins: Albumin, Globulin; Milk protein: Casein, rennet action on Cheese

2.37 Meat protein: Myoglobin Myoglobin is a water-soluble protein that stores oxygen for aerobic metabolism in the muscle. It consists of a protein portion and a nonprotein porphyrin ring with a central iron atom. The iron atom is an important player in meat color. The defining factors of meat color are the oxidation (chemical) state of the iron and which compounds (oxygen, water or nitric oxide) are attached to the iron portion of the molecule. Because muscles differ greatly in activity, their oxygen demand varies. Consequently different myoglobin concentrations are found in the various muscles of the animal. Also, as the animal gets older there is more myoglobin. A greater myoglobin concentration yields a more intense color. Muscle pigment concentration also differs among animal species. For example, beef has considerably more myoglobin than pork or lamb, thus giving it a more intense color.

2.38 Changes in colour during curing of meat:

Immediately after cutting, meat color is quite dark - beef would be a deep purplish-red. As oxygen from the air comes into contact with the exposed meat surfaces it is absorbed and binds to the iron. The surface of the meat blooms as myoglobin is oxygenated. This pigment, called oxymyoglobin, gives beef its bright cherry red color. It is the color consumers associate with freshness. Myoglobin and oxymyoglobin have the capacity to lose an electron (called oxidation) which turns the pigment to a brown color and yields metmyoglobin. Thus, myoglobin can change from a dark purple color to a bright red color simply from oxygenation or to a brown color by losing electrons. The pigments myoglobin, oxymyoglobin and metmyoglobin can be changed from one to the other, depending on the conditions at which the meat is stored. After cooking, a brown pigment called denatured metmyoglobin is formed, which normally cannot be changed to form another pigment. Oxymyoglobin, commonly known as the fresh meat color, is the most desirable color for fresh meats. Maintaining this color requires that the meat surface be free from any contamination which would cause a chemical reaction resulting in the formation of the brown pigment metmyoglobin. Also, oxygen must be available at a sufficient concentration in order to combine with the myoglobin to form oxymyoglobin. This reaction is reversible and dependent on the availability of oxygen, active enzymes and reducing compounds in the muscle. The change from myoglobin to oxymyoglobin and vice versa usually occurs quite readily. Similarly, the reaction that produces the brown meat metmyoglobin occurs quite easily, but the reverse of this is more difficult. In raw meat there is a dynamic cycle such that in the presence of oxygen the three pigments myoglobin, oxymyoglobin and metmyoglobin are constantly interconverted, all three forms are in equilibrium with one another.



2.39 Egg proteins:

Egg white proteins:

Albumen is the name for the clear liquid (also called the eggwhite or the glair/glaire) contained within an egg. The primary natural purpose of egg white is to protect the yolk and provide additional nutrition for the growth of the embryo (when fertilized). Egg white consists primarily of about 90% water into which is dissolved about 10% proteins (including albumins, mucoproteins, and globulins). Unlike the yolk, which is high in lipids (fats), egg white contains almost no fat, and carbohydrate content is less than 1%. Egg whites contain just over 50% of the protein in the egg. Ovalbumin is the most abundant protein in albumen. Classed as phosphoglycoprotein, during storage, it converts into s-ovalbumin (5% at the time of laying) and can reach up to 80% after six months of cold storage. Ovalbumin in solution is heat-resistant. Denaturation temperature is around 84°C, but it can be easily denatured by physical stresses. Conalbumin/ovotransferrin is a glycoprotein which has the capacity to bind the bi- and trivalent metal cations into a complex and is more heat sensitive than ovalbumin. At its isoelectric pH (6.5), it can bind two cations and assume a red or yellow color. These metal complexes are more heat stable than the native state. Ovomuroid is the major allergen from egg white and is a heat-resistant glycoprotein found to be a trypsin inhibitor. Lysozyme is a holoprotein which can lyse the wall of certain Gram-positive bacteria and is found at high levels in the chalaziferous layer and the chalazae which anchor the yolk towards the middle of the egg. Ovomucin is a glycoprotein which may contribute to the gel-like structure of thick albumen. The amount of ovomucin in the thick albumen is four times greater than in the thin albumen.

Egg yolk proteins:

The yolk makes up about 33% of the liquid weight of the egg; it contains about 60 Calories, three times the energy content of the egg white. All of the fat-soluble vitamins (A, D, E, and K)

are found in the egg yolk. Egg yolk is one of the few foods naturally containing vitamin D. The different yolk's proteins have distinct roles. Phosvitins are important in sequestering calcium, iron, and other cations for the developing embryo. Phosvitins are one of the most phosphorylated (10%) proteins in nature; the high concentration of phosphate groups provides efficient metal-binding sites in clusters. Lipovitellins are involved in lipid and metal storage, and contain a heterogeneous mixture of about 16% (w/w) noncovalently bound lipid, most being phospholipid. Yolks hold more than 90% of the calcium, iron, phosphorus, zinc, thiamine, vitamin B₆, folate, vitamin B₁₂, and pantothenic acid of the egg. In addition, yolks cover all of the fat-soluble vitamins: A, D, E, and K in the egg, as well as all of the essential fatty acids. A single yolk from a large egg contains roughly 22 mg of calcium, 66 mg of phosphorus, 9.5 micrograms of selenium, and 19 mg of potassium, according to the USDA.

Composition of Egg, Egg yolk and Egg white

Constituents	Whole Egg (%)	Egg yolk (%)	Egg white (%)
Water	73.7	51.1	87.6
Protein	12.9	16.0	10.9
Fat	11.5	30.5	Trace
Minerals	1.0	1.7	0.7
Vitamins	1.0	0.5	2.0

Major proteins of egg albumin

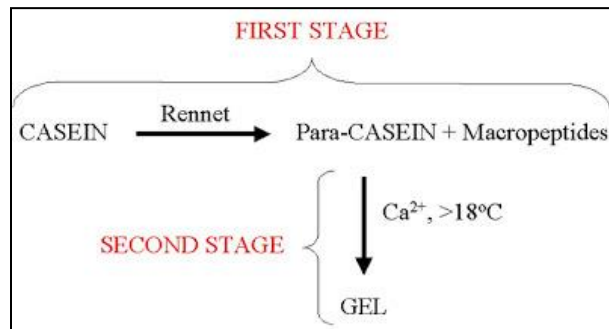
Protein	Amount in albumin (%)
Ovalbumin	54
Ovotransferrin (Conalbumin)	12
Ovomucoid	11
Lysozyme	3.4
Ovomucin	3.5
Ovoinhibitor	1.4
Ovoflavoprotein	0.8
Avidin	0.05

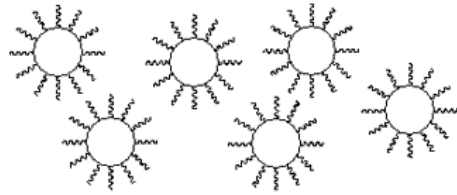
2.40 Milk protein: Casein

Caseins are phosphoproteins, M~20kD, synthesized in mammary gland „ Bovine casein particles are built up from four proteins „ Caseins have low levels of secondary and tertiary structures. In bovine milk about 90% of casein exists as macromolecular aggregates termed micelles, with molecular weight about 10⁸ kD and mean diameter of 200nm. Contain on dry weight basis 94% of protein and 6% of minerals. Micelles are hydrated containing up to 3.3 g water per gram of

protein. Micelles are porous occupying about 4 ml/g. Native casein micelles are quite stable during technological treatment

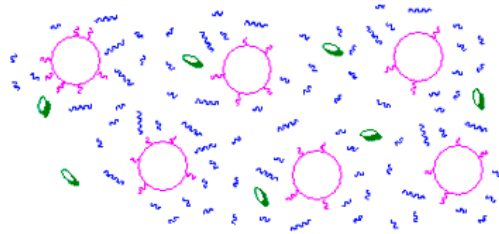
Rennet action on Cheese: Rennet coagulation follows the specific hydrolyses of micelle stabilizing surface layer during this step glucomacropptide is lost „ At the natural pH of milk (6.7), about 80% of κ -casein must be cleaved to permit aggregation of the micelles „ After losing its water-soluble tail κ -casein can no longer keep the casein particles separated, the diameter of casein micelles is reduced 7-10 nm. Optimum coagulation temperature of milk for most cheese varieties is 30-32 °C. At the temperature less than 30°C the gel is weak and difficult to cut without excessive yield loss. At temperatures less than 20°C the second stage of renneting, coagulation, do not occur, but the primary stage goes to completion.





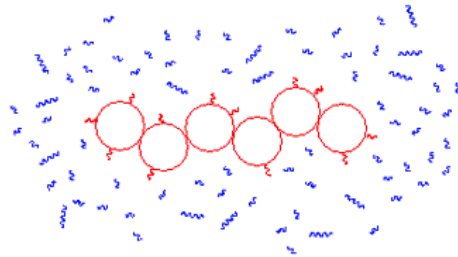
κ -casein micelles
(water soluble due to
hydrophilic "hairy" coating)

↓
*proteolytic enzyme (protease) with milk clotting
ability specifically hydrolyses "hairy" stabilizing
coating on κ -casein*



proteolytic enzyme
acidic glycopeptide
(water soluble)
para- κ -casein
(less water soluble)

↓
*the loss of the stabilizing "hairy" results in a destabilization of the
casein micelles and subsequent calcium promoted aggregation
and gel formation*



aggregated micelles

Lecture 1

Fats: Sources; Classifications; Fatty acids:

3.1. Definition of Lipid: Lipids comprise a group of naturally occurring molecules that include fats, waxes, sterols, fat-soluble vitamins (such as vitamins A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others. The main biological functions of lipids include storing energy, signaling, and acting as structural components of cell membranes. Lipids have applications in the cosmetic and food industries as well as in nanotechnology.

3.2. Different Types of Lipids:

- I. **Simple lipids or homolipids:** Fat and oils and waxes.
- II. **Compound lipids:** Phospholipids, Glycolipids.
- III. **Derived lipids**

3.3. Triglycerides:

Triglycerides contain three fatty acids molecules attached with one glycerol molecule. We can classify them in fats and oils.

3.3.1. Fats: They are solid at room temperature.

Sources:

- **Vegetable sources:** Vegetable ghee, vanaspati, margarine etc.
- **Animal sources:** Cheese, butter, beef fat etc.

3.3.2. Oils: They are liquid at room temperature.

Sources:

- **Vegetable sources:** Mastered oil, coconut oil, sunflower oil, soy-bean oil etc.

Examples:

Name	Formula	C:D
Caprylic acid	CH ₃ (CH ₂) ₆ COOH	8:0
Capric acid	CH ₃ (CH ₂) ₈ COOH	10:0
Lauric acid	CH ₃ (CH ₂) ₁₀ COOH	12:0
Myristic acid	CH ₃ (CH ₂) ₁₂ COOH	14:0
Palmitic acid	CH ₃ (CH ₂) ₁₄ COOH	16:0
Stearic acid	CH ₃ (CH ₂) ₁₆ COOH	18:0
Arachidic acid	CH ₃ (CH ₂) ₁₈ COOH	20:0
Behenic acid	CH ₃ (CH ₂) ₂₀ COOH	22:0
Lignoceric acid	CH ₃ (CH ₂) ₂₂ COOH	24:0
Cerotic acid	CH ₃ (CH ₂) ₂₄ COOH	26:0

3.4.1.2. MUFA: Monounsaturated fatty acids (abbreviated MUFAs, or more plainly monounsaturated fats) are fatty acids that have one double bond in the fatty acid chain.

Name	Formula	C:D
Oleic acid	CH ₃ (CH ₂) ₇ CH=CH(CH ₂) ₇ COOH	18:1 n-9
Palmitoleic acid	CH ₃ (CH ₂) ₅ CH=CH(CH ₂) ₇ COOH	16:1 n-7
Vaccenic acid	C ₁₈ H ₃₄ O ₂	18:1 n-7

3.4.1.3. PUFA: Polyunsaturated fatty acids (PUFAs) are fatty acids that contain more than one double bond in their backbone. This class includes many important compounds, such as essential fatty acids.

- **Omega 3 fatty acids:** They have their 1st double bond at the 3rd carbon atom from the omega or methyl end.
- **Omega 6 Fatty Acids:** They have their 1st double bond at the 6th carbon atom from the omega or methyl end.
- **Omega 9 fatty acid:** Here the 1st double bond is present at the 9th carbon atom from the methyl or omega group. Example: Oleic acid (MUFA)

Note: Omega 3 and omega 6 fatty acids are known as essential fatty acids.

Examples of PUFA :

Common Name	Chemical Name	C:D	Omega
Linoleic acid	9,12-octadecadienoic acid	18:2	6
Linolenic acid	9,12,15	18:3	3
Alpha-linolenic acid	9,12,15-octadecatrienoic acid	18:3	3
Arachidonic acid	5,8,11,14-eicosatetraenoic acid	20:4	6
Docosapentaenoic acid	4,7,10,13,16-docosapentaenoic acid	22:5	6
Eicosapentaenoic acid	5,8,11,14,17-eicosapentaenoic acid	20:5	3
Docosapentaenoic acid	4,7,10,13,16-docosapentaenoic acid	22:5	6

3.5. Functions of Omega-3 and omega-6 in the body:

- Both omega-3 (ω -3) and omega-6 (ω -6) fatty acids are important components of cell membranes
- They are precursors to many other substances in the body such as those involved with regulating blood pressure and inflammatory responses.
- There is increasing support for omega-3 fatty acids in protecting against fatal heart disease
- They have anti-inflammatory effects
- There is also growing interest in the role of omega-3 fatty acids in the prevention of diabetes and certain types of cancer.

3.6. Essential Fatty Acids:

The human body is capable of producing all the fatty acids it needs, except for two: **linoleic acid (LA)**, an omega-6 fatty acid, and **alpha-linolenic acid (ALA)**, an omega-3 fatty acid. These have to be consumed from the diet and are therefore termed “essential fatty acids”. Both of these fatty acids are needed for growth and repair, but can also be used to make other fatty acids (e.g. arachidonic acid (AA) is formed from LA). However, as conversion to the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is limited, it is recommended that

sources of these are also included in the diet. ALA and LA are found in plant and seed oils. Although the levels of LA are usually much higher than those of ALA, rapeseed oil and walnut oil are very good sources of the latter. EPA and DHA are found in oily fish (e.g., salmon, mackerel, and herring). AA can be obtained from animal sources, such as meat and egg yolk.

3.6.1. Intakes:

Recommended intakes of omega-3 vary by country from 0.5 to 2% of energy: recommended intakes of ALA are between 0.6 and 1.2% of energy or 1-2 g/day. A study of dietary intakes of various types of fat found that actual intakes of ALA vary from around 0.6 g/d (France and Greece) to 2.5 g/d (Iceland) in men and 0.5 g/d (France) to 2.1 g/d (Denmark) in women.⁴Intakes are too low in most cases and increasing our consumption of omega-3 rich foods would be beneficial to most diets. This may be achieved for example by eating fatty fish once or twice a week and by occasionally replacing sunflower oil with rapeseed oil.

Lecture 3

Physico chemical and functional properties: Rancidity : Definition, types of rancidity of fats and oils (hydrolytic and oxidative rancidity);

3.7.Rancidity:

Rancidity is a term generally used to denote unpleasant odours and flavours in foods resulting from deterioration in the fat or oil portion of a food. Three different mechanisms of rancidity may occur. These are oxidative, hydrolytic, and ketonic.

- **Oxidative rancidity** of fats such as lard, shortenings, salad and cooking oils refers to the undesirable odors and flavors which develop when such products are exposed to the oxygen in the air. Products containing these fats, including but not limited to food products such as fish, poultry, meat, frozen vegetables and dry milk can become rancid as the fats in the products react to air. The poly-unsaturated fatty acid portions of these foods react with oxygen to form peroxides. The peroxides decompose to yield a complex of mixtures, including aldehydes, ketones, and other volatile products. These products are responsible for "rancid" odors and flavors. It is important to note that fish contain highly unsaturated (poly-unsaturated and mono-unsaturated) fatty acids which make some fish products particularly susceptible to oxidative deterioration. Highly saturated products, such as butter, are not as prone to oxidative rancidity due to the absence of polyunsaturated fatty acid compounds. These products also tend to be more solid at room temperature.
- **Hydrolytic rancidity** refers to the odor that develops when triglycerides are hydrolyzed and free fatty acids are released. This reaction of lipid with water sometimes requires a catalyst, but results in the formation free fatty acids and salts from free fatty acids (soaps). In particular, short chain fatty acids, such as common butter fats, are odorous.

- **Ketonic rancidity:** Some moulds (*Penicillium* and *Aspergillus* spp.) attack fats containing short-chain fatty acids and produce ketones with a characteristic odour and taste. Butter, coconut, and palm kernel oils are most susceptible.

3.7.1. Factors Influencing Fat Oxidation:

- **Temperature:** The rate of fat oxidation is highly dependent on temperature. Considerable improvement in storage stability can therefore be gained by lowering the storage temperature. As an example, it has been found that the storage time for frozen raw, lean meat can be extended approximately by 3 times by lowering the temperature from 5 to -13°F (-15 to -25°C). Temperature fluctuations during storage should also be minimized.
- **Oxygen:** Oxygen in the air may be displaced by an inert gas such as nitrogen or carbon dioxide to retard oxidative rancidity, or the products may be packed under vacuum. These methods require the use of packaging materials with low oxygen permeability.
- **Type of fat:** In general, the softer the fat, the more unsaturated are the fatty acids and the more susceptible they are to oxidation and oxidative rancidity. However, vegetable fats, although unsaturated, are usually more stable than animal fats because they contain natural antioxidants. The most common antioxidant found in vegetable fats is vitamin E. Coconut oil is more saturated and, therefore, more stable than most other vegetable oils. Fish oils are highly unsaturated, and therefore more susceptible to oxidative rancidity and off odors and flavors. The process of Hydrogenation, which involves the addition of hydrogen to unsaturated bonds, effectively hardens fat and increases its saturation, therefore providing additional resistance to oxidation. It is important to note that the susceptibility to oxidation of a product may be dependent on factors other than the degree of un-saturation of the fat. One important factor influencing oxidation, other than level of saturation, is the possibility for contact between the fat and pro-oxidants, antioxidants, or oxygen. For this reason, ground or minced flesh products are less stable than whole flesh products, since the muscle surface area has been increased through mincing or grinding, and oxygen has been mixed throughout the product as part of the mechanical process. For this reason, further processed products may be more prone to oxidative rancidity than whole-muscle cuts.
- **Light:** Packages that exclude light can be used to protect the products against fat oxidation.
- **Metals:** Metals such as copper, iron, manganese, and chromium increase rate of fat oxidation. As a result, the preferred storage containers are steel drums, tin, or nonmetallic materials such as plastic. Stainless steel is commonly used in processing plants so as to avoid excessive contact with metals that increase fat oxidation. It is important to note that water with trace metal is often a cause of rancidity in food products.
- **Products from fat oxidation:** Traces of oxidized fat in ingredients can accelerate oxidative rancidity in the remainder of the products. Blending oxidized products with un-oxidized products is not recommended. Steam treatment under vacuum conditions has been effective in removing products of deterioration (odorous substances) from some oils and fats.

Lecture 4

Reversion of fats Antioxidants: Definition, examples, roles.

3.8. Reversion of oil:

It is the breakdown (oxidation) of an essential fatty acid, linolenic acid, found in certain vegetable oils, leading to an undesirable flavor change prior to the start of actual rancidity. Therefore, before the actual decay of the oil one can "taste" the difference and predict that the oil may run bad in future. A reversed oil/fat once detected can help save us from consuming a potentially bad fat/oil. That's because a rancid oil can have damaging health effects such as cancer, heart disease etc. Oils which are resistant to reversion are cottonseed and corn oil.

3.9. Antioxidants

Food antioxidants are compounds that increase the resistance of fats to oxidation and consequent deterioration or rancidity. Only specifically approved antioxidants are able to be added to lard, tallow, and other foods susceptible to rancidity. In the United States, authorization of antioxidants for use in meat products is a responsibility of the Food Safety Inspection Service (FSIS) of the United States Department of Agriculture (USDA). Antioxidants used in dairy products, salad dressings and oils are regulated by the Food and Color Additives Division of the U. S. Food and Drug Administration (FDA). Antioxidant inclusion is restricted to specific limits and must be declared on product labels. Some of the approved antioxidants are butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), propyl gallate, and tocopherols. These primary antioxidants are often used in combination with citric or phosphoric acid. Use of one or more of the primary antioxidants in combination with one of the acids is common because combinations are much more effective than single antioxidants. Many food grade combinations are possible. It is important to note that antioxidants cannot be expected to stop rancidity. Their effectiveness lies only in slowing down the rate of oxidation and varies with the antioxidant combination used and with the food product to be protected. Natural antioxidants, such as those contained in some spices, such as rosemary, sage, and marjoram, have met acceptance for the retardation of rancidity in meat products. These and other natural antioxidants not only retard the warmed over flavor (WOF) in precooked meat products, but provide an agreeable aromatic aroma and flavor. Some spice extracts, particularly rosemary, are prepared primarily for their antioxidant activity and do not include strong flavor components. Natural antioxidants from fruit products, including but not limited to pear and plum extracts, have also been shown to effectively reduce oxidative rancidity in ground meat products while providing additional sources of nutrients and flavor.

Lecture 5

Hydrogenation of oils. Saponification, inter-esterification

3.10. Hydrogenation of oil:

3.10.1. Definition: Hydrogenation is the process of heating an oil and passing hydrogen bubbles through it. The fatty acids in the oil then acquire some of the hydrogen, which makes it more dense. If you fully hydrogenate, you create a solid (a fat) out of the oil. But if you stop part way, you a semi-solid partially hydrogenated oil that has a consistency like butter, only it's a lot cheaper.

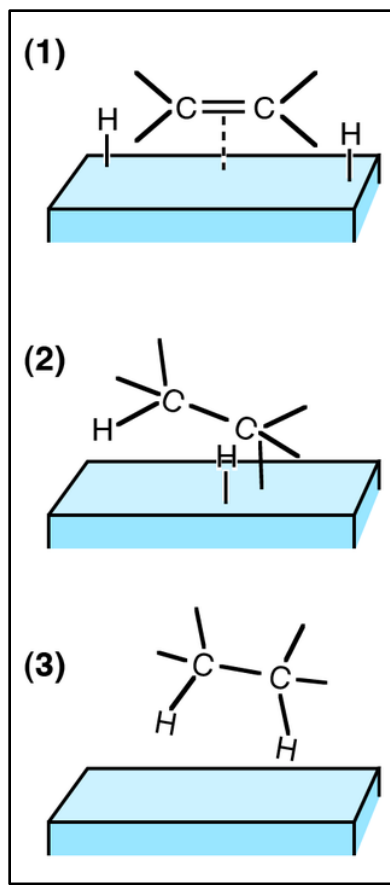
Because of that consistency, and because it is cheap, it is a big favorite as a butter-substitute among "food" producers. It gives their products a richer flavor and texture, but doesn't cost near as much as it would to add butter.

Catalysts are required for the reaction to be usable; non-catalytic hydrogenation takes place only at very high temperatures. Hydrogenation reduces double and triple bonds in hydrocarbons.

3.10.2. Hydrogen sources: For hydrogenation, the obvious source of hydrogen is H_2 gas itself, which is typically available commercially within the storage medium of a pressurized cylinder. The hydrogenation process often uses greater than 1 atmosphere of H_2 , usually conveyed from the cylinders and sometimes augmented by "booster pumps". Gaseous hydrogen is produced industrially from hydrocarbons by the process known as steam reforming. For many applications, hydrogen is transferred from donor molecules such as formic acid, isopropanol, and dihydroanthracene. These hydrogen donors undergo dehydrogenation to, respectively, carbon dioxide, acetone, and anthracene. These processes are called transfer hydrogenations.

3.10.3. Catalysts: Generally Platinum, palladium, rhodium, and ruthenium are used. They operate the process at lower temperatures and lower pressures of H_2 .

3.10.4. Mechanism of Hydrogenation process:



- (1) The reactants are adsorbed on the catalyst surface and H_2 dissociates.
- (2) An H atom bonds to one C atom. The other C atom is still attached to the surface.
- (3) A second C atom bonds to an H atom. The molecule leaves the surface.

3.11. Industrial use of Hydrogenated oil:

Vanaspati or shortening agent

Confectionary fats (hard butter)

Margarine

3.12. Partial hydrogenation: Generally when vegetable oils are solidifying by using hydrogenation process, partial hydrogenation or incomplete hydrogenation may occur. Most of the double bonds are removed in the process, which elevates the melting point of the product.

In most oils with a high content of C18 unsaturated acids the possible reactions are:

Linolenic to linoleic acid

Linoleic to oleic acid

Oleic to stearic acid

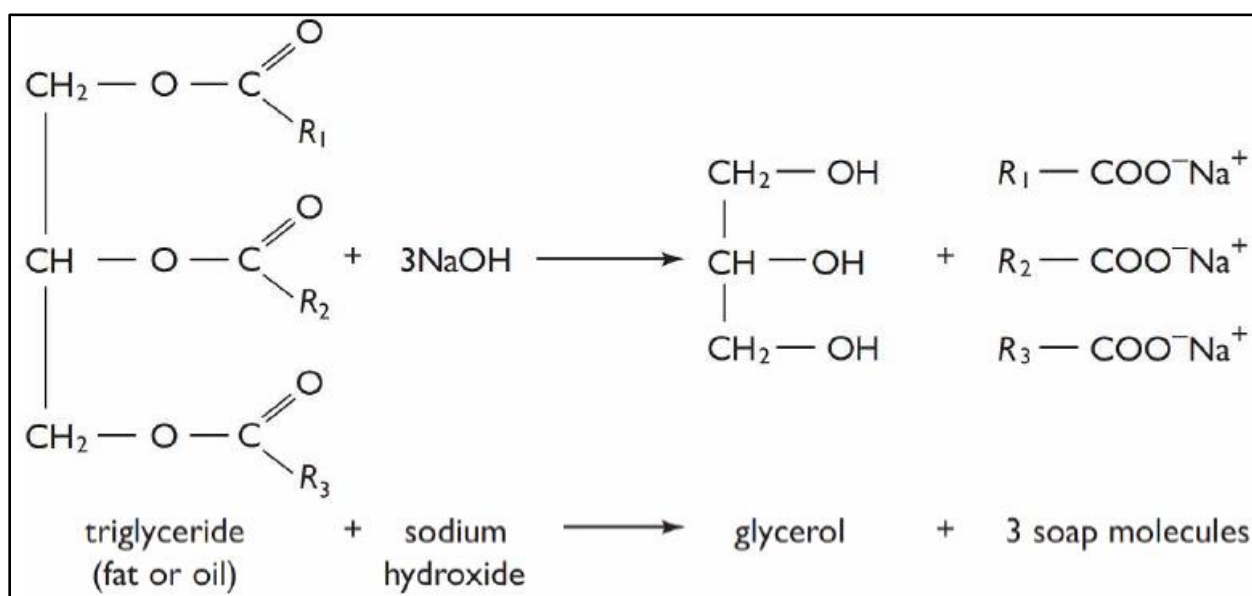
3.13. Side effects of partial hydrogenation process: A side effect of incomplete hydrogenation having implications for human health is the isomerization of some of the remaining unsaturated carbon bonds. The cis configuration of these double bonds predominates in the unprocessed fats in most edible fat sources, but incomplete hydrogenation partially converts these molecules to trans isomers, which have been implicated in circulatory diseases including heart disease.

3.14: Saponification: Usually, a process by which triglycerides are reacted with sodium or potassium hydroxide to produce glycerol and a fatty acid salt, called 'soap'. When sodium hydroxide is used, a hard soap is produced. Using potassium hydroxide results in a soft soap.

Lipids that contain fatty acid ester linkages can undergo hydrolysis. This reaction is catalyzed by a strong acid or base. Saponification is the alkaline hydrolysis of the fatty acid esters.

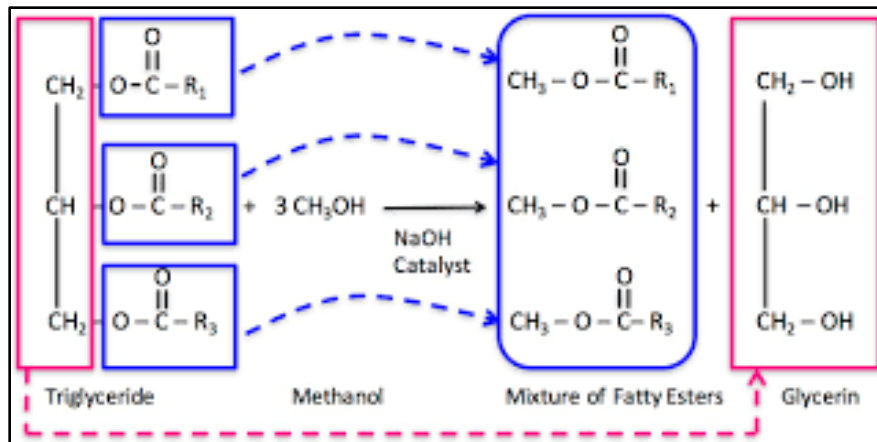
Example: The chemical reaction between any fat and sodium hydroxide is a saponification reaction.

triglyceride + sodium hydroxide (or potassium hydroxide) → glycerol + 3 soap molecules



3.15. Inter-esterification of oil:

Definition of inter-esterified fat: Interesterified fat is a type of oil where the fatty acids have been moved from one triglyceride molecule to another. This is generally done to modify the melting point, slow rancidification and create oil more suitable for deep frying or making margarine with good taste and low saturated fat content.



In the food industry, interesterification can be carried out using a chemical catalyst or an enzyme. Sodium methoxide is generally used as a catalyst in chemical esterification, while lipases are used in enzymatic esterification. Chemical interesterification is a random reaction while enzymatic interesterification can be random or regiospecific.

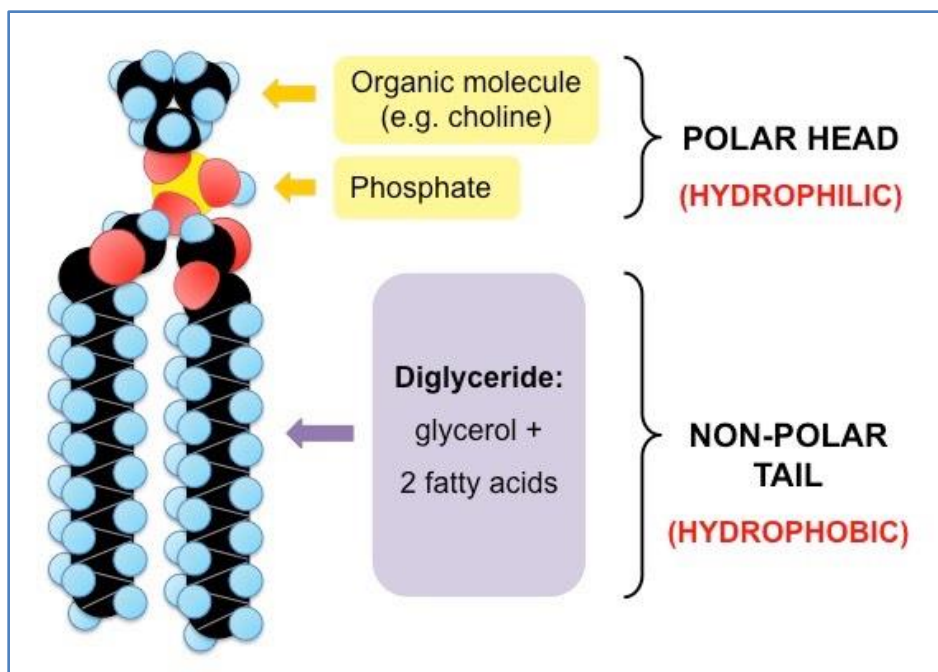
Lecture 6

Lipids of biological importance like phospholipids: lecithin cephalin

3.16: Phospholipid: Phospholipids are a class of lipids that are a major component of all cell membranes. They can form lipid bilayers because of their amphiphilic characteristic. The structure of the phospholipid molecule generally consists of two hydrophobic fatty acid "tails" and a hydrophilic "head" consisting of a phosphate group. The two components are joined together by a glycerol molecule. The phosphate groups can be modified with simple organic molecules such as choline.

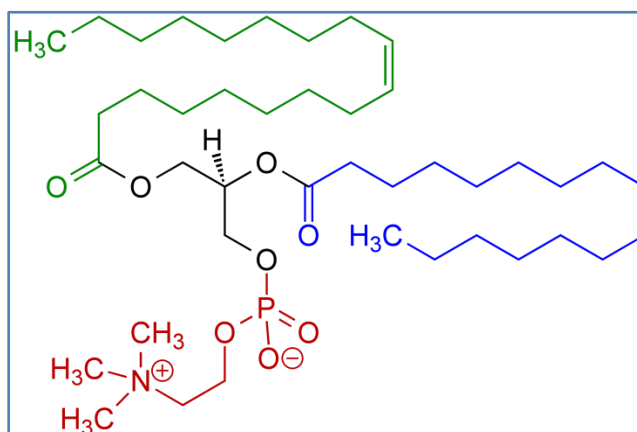
An amphiphile is a term describing a chemical compound possessing both hydrophilic (water-loving, polar) and lipophilic (fat-loving) properties. The phospholipid head contains a negatively charged phosphate group and glycerol; it is hydrophilic (attracted to water). The phospholipid tails usually consist of 2 long fatty acid chains; they are hydrophobic and are repelled by water. When placed in water, phospholipids form a variety of structures depending on the specific properties of the phospholipid with tails typically aggregating to minimize interactions with water molecules. These specific properties allow phospholipids to play an important role in the phospholipid bilayer.

Examples: Lecithin, Cephalin etc.



3.17. Lecithin: Lecithin is present in animal and plant tissues, which are amphiphilic - they attract both water and fatty substances (and so are both hydrophilic and lipophilic), and are used for smoothing food textures, dissolving powders (emulsifying), homogenizing liquid mixtures, and repelling sticking materials.

Lecithins are usually phospholipids, composed of phosphoric acid with choline, glycerol or other fatty acids usually glycolipids or triglyceride. Glycerophospholipids in lecithin include phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine, and phosphatidic acid.



(Red - choline and phosphate group; Black - glycerol; Green - unsaturated fatty acid; Blue - saturated fatty acid)

In food industry generally egg lecithin and soy lecithin are used as emulsifier.

3.18. Cephalin: Cephalin are closely associated with lecithin in animal tissues. They are also identified from soy-bean oil. These are similar in structure to the lecithin except that the biochemical precursor of ethanolamine.

Accordingly, two types of cephalins are recognized phosphatidylethalamine and phosphatidyle serine. This primary amino group of ethalamine is a weaker base than the ammonium group of choline, the cephalins are more acidic than lecithin. Moreover, competitively cephalins are less soluble in alcohol than lecithin.

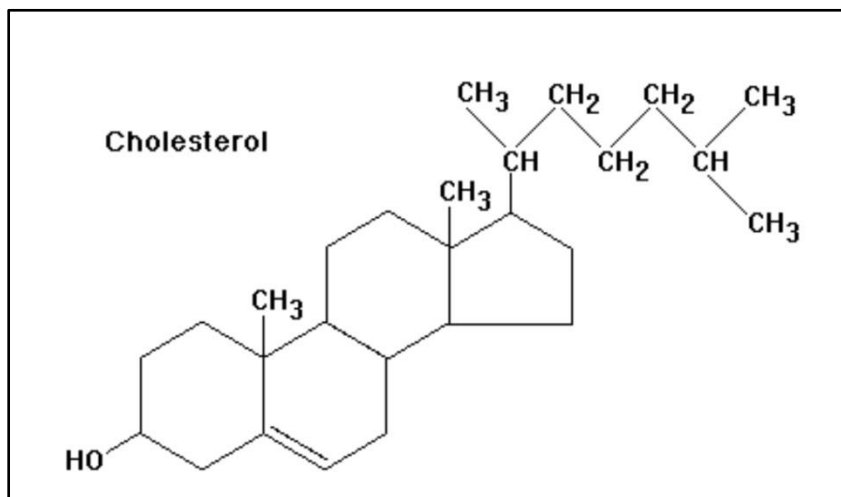
Lecture 7

Sterols: Cholesterol; Polymorphism of fat;

3.19. Sterols: Sterols are any of a group of solid, mostly unsaturated, polycyclic alcohols, as cholesterol and ergosterol, derived from plants or animals.

3.20: Cholesterol: Cholesterol, a type of lipid molecule, and is biosynthesized by all animal cells, because it is an essential structural component of all animal cell membranes; essential to maintain both membrane structural integrity and fluidity. Cholesterol enables animal cells to dispense with a cell wall (to protect membrane integrity and cell viability), thereby allowing animal cells to change shape rapidly and animals to move (unlike bacteria and plant cells, which are restricted by their cell walls).

In addition to its importance for animal cell structure, cholesterol also serves as a precursor for the biosynthesis of steroid hormones, bile acid, and vitamin D. Cholesterol is the principal sterol synthesized by all animals. In vertebrates, hepatic cells typically produce the greatest amounts. It is absent among prokaryotes (bacteria), although there are some exceptions, such as *Mycoplasma*, which require cholesterol for growth.



3.20.1. Physiological functions of Cholesterol:

- Cell membrane synthesis – Cholesterol helps to regulate membrane fluidity over the range of physiological temperatures. It has a hydroxyl group that interacts with the polar head groups of the membrane phospholipids and sphingolipids. These exist along with nonpolar fatty acid chain of the other lipids. Cholesterol also prevents the passage of protons (positive hydrogen ions) and sodium ions across the plasma membranes.
- Cell transporters and signalling molecules – The cholesterol molecules exist as transporters and signalling molecules along the membrane. Cholesterol also helps in nerve conduction.
- Cholesterol in the myelin sheaths – The nerve cells are covered with a protective layer or myelin sheath. The myelin sheath is rich in cholesterol. This is because it is derived from compacted layers of Schwann cell membrane. It helps in providing protection, insulation and allows more efficient conduction of nerve impulses.
- Role inside the cells – Within the cells, cholesterol is the precursor molecule in several biochemical pathways. For example, in the liver, cholesterol is converted to bile, which is then stored in the gallbladder. Bile is made up of bile salts. This helps in making the fats more soluble and helps in their absorption. Bile salts also aid in absorption of fat soluble vitamins like Vitamins A, D, E and K.
- Hormones and Vitamin D - Cholesterol is an important precursor molecule for the synthesis of Vitamin D and the steroid hormones like Corticosteroids, Sex-steroids (Sex hormones like Estrogen, Progesterone and Testosterone etc.)

3.21: Polymorphism of fat: Triglycerides are three-fold esters of glycerol and fatty acids. Triglycerides can be divided into two classes depending on the fatty acid composition. Triglycerides having only one type of fatty acid are called monoacid Triglycerides, and those having two and three types of fatty acids are, respectively, called diacid and triacid Triglycerides, and both are categorized as mixed-acid Triglycerides. Almost all natural fats and oils are mixed-acid Triglycerides.

Polymorphism of fat exhibit a number of crystalline structure with different packaging. Fats & triglycerides occur in any one of three basic types: α (alpha), β' (beta prime) and β (beta). All fats have an α polymorph; some are β' stable; some are β stable. Transitions go from α to β' to β , in that order, which is the order of increasing stability.

Lecture 8

Quantitative tests: Saponification number, acid value, iodine value, peroxide value Reichert-Meissl number, Polenske value.;

3.22. Saponification number: The saponification number is the number of milligrams of potassium hydroxide required to neutralize the fatty acids resulting from the complete hydrolysis of 1g of fat.

Significance: It gives information concerning the character of the fatty acids of the fat- the longer the carbon chain; the less acid is liberated per gram of fat hydrolysed. It is also considered as a measure of the average molecular weight (or chain length) of all the fatty acids present. The long chain fatty acids found in fats have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat and therefore high molecular weight.

3.23: Acid value: It is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1g of fat.

Significance: It is a measure of the free fatty acids (FFA) present in the fat or oil. An increment in the amount of FFA in a sample of oil or fat indicates hydrolysis of triglycerides.

3.24. Iodine Value: Iodine value or number is the number of grams of iodine consumed by 100g of fat.

Significance: A higher iodine value indicates a higher degree of unsaturation.

3.25. Peroxide Value: The peroxide value is defined as the amount of peroxide oxygen per 1 kilogram of fat or oil.

Significance: The Peroxide Value (PV) of an oil or fat is used as a measurement of the extent to which rancidity reactions have occurred during storage. Other methods are available but peroxide value is the most widely used.

The molecular structure of fats and oils play a role in autoxidation. Oils with a high degree of unsaturation are most susceptible to autoxidation. The best test for autoxidation (oxidative rancidity) is determination of the peroxide value, as peroxides are intermediates in the autoxidation reaction. Autoxidation is a reaction involving oxygen that leads to deterioration of fats and oils which form off-flavours and off-odours. Peroxide value, which is the concentration of peroxide in an oil or fat, is useful for assessing the extent to which spoilage has occurred.

3.26: Reichert-Meissl number: It is an index of the volatile acid content of a fat; the number of milliliters of 0.1 N KOH required to neutralize the soluble volatile fatty acids in 5 g of fat that has been saponified, acidified to liberate the fatty acids, and then steam-distilled.

3.27. Polenske value: It is the number of milliliters of 0.1 N KOH required to neutralize insoluble fatty acids obtained from 5 gm of fats.

Module 4

8L

Lecture 1

Pigments: Definition; Functions, Classifications

4.1 Definition: A pigment is a material that changes the color of reflected or transmitted light as the result of wavelength-selective absorption. This physical process differs from fluorescence, phosphorescence, and other forms of luminescence, in which a material emits light. Pigments are used for coloring paint, ink, plastic, fabric, cosmetics, food, and other materials. Most pigments used in manufacturing and the visual arts are dry colorants. In biology, a pigment is any colored material of plant or animal cells. Many biological structures, such as skin, eyes, fur, and hair contain pigments (such as melanin). Animal skin coloration often comes about through specialized cells called chromatophores, which animals such as the octopus and chameleon can control to vary the animal's color. Pigmentation in organisms serves many biological purposes, including camouflage, mimicry, aposematism (warning), signalling, photosynthesis (in plants), as well as basic physical purposes such as protection from sunburn.

4.2 Functions: The primary function of pigments in plants is photosynthesis, which uses the green pigment chlorophyll along with several red and yellow pigments that help to capture as much light energy as possible. Other functions of pigments in plants include attracting insects to flowers to encourage pollination. Plant pigments include a variety of different kinds of molecule, including porphyrins, carotenoids, anthocyanins and betalains. All biological pigments selectively absorb certain wavelengths of light while reflecting others.

4.3 Classifications:

The principal pigments in plants are-

1. Chlorophyll

2. Carotenoids
3. Xanthophylls
4. Anthocyanins
5. Betalains

PHOTOSYNTHETIC PIGMENTS : THEIR DISTRIBUTION AND LIGHT ABSORPTION

Pigment	Distribution	Light absorption (In nm)
1. Chlorophylls		
Chlorophyll-a	All photosynthesizing plants except bacteria.	429,410,660
Chlorophyll-b	Higher plants and green algae.	453,430,642
Chlorophyll-c	Diatoms, dinoflagellates and brown algae.	645
Chlorophyll-d	In some red algae	-
Chlorophyll-e	In <i>Tribonema</i> and zoospores of <i>Vaucheria</i>	-
Bacteriochlorophyll-a	Purple and green bacteria	-
Bacteriochlorophyll-b	In a strain of purple bacterium <i>Rhodospseudomonas</i> .	-
Bacteriochlorophyll-c, d & e (Chlorobium chlorophyll or Bacterioviridin)	Green bacteria	
Bacteriochlorophyll-g	Heliobacteria	
2. Carotenoids		
Carotenes	Mostly in algae and higher plants	
Xanthophylls (Carotenols)	Mostly in algae and higher plants	
3. Phycobilins		
Phycoerythrins	In blue-green and red algae	490,546,576
Phycocyanins	In blue-green and red algae	618

4.4 Food Sources of plant pigments and their health benefits:

Flavonoids	Food Sources	Health Benefits
<i>Catechins, Flavanones, Flavones, Isoflavones and Flavonols</i>	Widely found in fruits and vegetables, mainly in berries, citrus fruits, broccoli, cabbage, cucumber, green peppers	Preventative against cancers, and infections
<i>Anthocyanins</i>	Red and blue pigments e.g. cherries, strawberries, blueberries	Lessen exercise-induced oxidative stress
<i>Proanthocyanidins</i>	Found in Berries e.g. cranberry, blueberry, black berry and black raspberry	Reduce the risk of UTI's, preventing bacterial infections and improving dental health
<i>Quercetin</i>	Mainly contained in apple skin, red onion and red grape	Prevent LDL oxidation, reducing damage to DNA, helping to reduce CHD
Carotenoids	Food Sources	Health Benefits
<i>Beta-carotene</i>	Orange-coloured fruit and vegetables and dark leafy green vegetables e.g. carrots, pumpkin, sweet potato, apricot, spinach, kale	Lower risk of cancer and heart disease; protect the skin from UV rays.
<i>Lycopene</i>	Red-coloured fruits e.g. tomato, watermelon, pink grapefruit	Preventive against CHD; protective against carcinogens; preventive or delaying against certain types of cancer
<i>Lutein & Zeaxanthin</i>	Green and yellow leafy vegetables, green fruits e.g. spinach, kiwi, zucchini	Reduce the risk of age-related macular degeneration and cataracts
Glucosinolates	Food Sources	Health Benefits
<i>Indoles</i>	Vegetables such as cabbage, kale, broccoli	Anti-carcinogenic properties, and detoxification ability
<i>Isothiocyanates</i>	Cruciferous vegetables such as watercress, broccoli and radish	Reduce the risk of lung cancer, preventative against organ tumours

Lecture 2

Chlorophyll: Sources, Functions, Classifications, Changes during processing

4.5 Definition: Chlorophyll is the primary pigment in plants; it is a chlorin (large heterocyclic aromatic ring) that absorbs yellow and blue wavelengths of light while reflecting green. It is the presence and relative abundance of chlorophyll that gives plants their green color.

4.6 Sources: All land plants and green algae possess two forms of this pigment: chlorophyll *a* and chlorophyll *b*. Kelps, diatoms, and other photosynthetic heterokonts contain chlorophyll *c* instead of *b*, while red algae possess only chlorophyll *a*. All chlorophylls serve as the primary means plants use to intercept light in order to fuel photosynthesis.

4.7 Classifications:

Generally exists in two types-

Chlorophyll *a*: Involved in light reactions.

Chlorophyll b: assists in capturing light energy.

4.8 Chlorophyll: Changes during processing

Green colour in vegetables is due to the presence of Chlorophyll pigments. This is the universal pigment that carries out photosynthesis in plants, algae and photosynthetic bacteria. Chlorophyll exists in two forms: "Chlorophyll a" and "Chlorophyll b". Chlorophyll a ($C_{55}H_{72}MgN_4O_5$) is a complex molecule with a molecular weight of 893.49. It is a tetrapyrrole structure consisting of phytol, a higher alcohol and a Mg^{2+} ion chelated in the centre. Chlorophyll a content is more in green food items. However, preservation of green colour is important in food industry. Persistence of natural green colour in food is an indication of a healthy food item to consumers.

The green colour in different food items such as dehydrated vegetables, green tea leaves, green chili sauce, porridge, olives, fermented products shows different green shade. This results from various processing conditions used in cooking or preserving of the food. This green pigment which is naturally present is converted to various forms when exposed to different conditions. Concentration of Chlorophyll present, presence of phenolic compound which enhances enzymatic browning, entrapment of the pigment within cellulose, amount of acids present are also key factor for determining the specific shade of green apart from structural changes that occur in chlorophyll. Chlorophyll is naturally present linked to a lipoprotein which protects it from acid digestion. These organic acids present in cell vacuoles are denatured during food processing. Phytol which is a higher alcohol in Chlorophyll makes the Chlorophyll molecule insoluble in water. In maceration of green leaves, green pigment and the Chlorophyllase enzyme present inside the cell comes out. Chlorophyllase enzyme removes the phytol group from the pigment producing Chlorophyllide, which is water soluble. Chlorophyllide is further degraded into purpurins in the presence of acids and oxygen which results in loss of green colour. Chlorophyllide is converted to Pheophorbide a in the presence of acids. Pheophorbide a is olive brown in colour. This also can be converted to Purpurins by oxidation.

Many food processing methods, involves heating the vegetables. The degree of heating differs when steaming, frying, tempering etc. This affects the green colour of the food. When heating green vegetables, pheophytinization can take place in which the Mg^{2+} ions are replaced by H^+ ions producing pheophytin a from Chlorophyll a. Pheophytin a can be converted to purpurins and Pheophorbide under different conditions. In food processing, using high temperatures for a short time is effective in preserving green colour. Blanching is a good method to preserve green colour as it inactivates the enzyme system of the green vegetables. In drying, sun drying is not effective in retaining green colour. It enhances photochemical oxidation of Chlorophyll pigment.

Amount of phenolic compounds in vegetables indirectly affect the shade of green in food. These phenolic compounds are the substrate to enzymatic browning reactions. Product of enzymatic browning is a brown colour pigment known as melanin. This brown product masks the green colour that is naturally present. This occurs when the vegetables are cut, macerated, damaged which expose internal tissues to oxygen. Photochemical oxidation also results in change of natural green colour present in vegetables. This plays a significant role in selecting of packaging materials for marketing of green vegetables. Higher oxygen concentration and light increase the rate of photochemical oxidation. Therefore, it's a must to select packaging material with higher oxygen and light barrier properties.

Lecture 3

Carotenoids: Sources, Functions, Classifications

4.9 Definition: Carotenoids are a class of more than 600 naturally occurring pigments synthesized by plants, algae, and photosynthetic bacteria. These richly colored molecules are the sources of the yellow, orange, and red colors of many plants. Carotenoids are red, orange, or yellow tetraterpenoids.

4.10 Functions: During the process of photosynthesis, they have functions in light-harvesting (as accessory pigments), in photoprotection (energy dissipation via non-photochemical quenching as well as singlet oxygen scavenging for prevention of photooxidative damage), and also serve as protein structural elements. In higher plants, they also serve as precursors to the plant hormone abscisic acid.

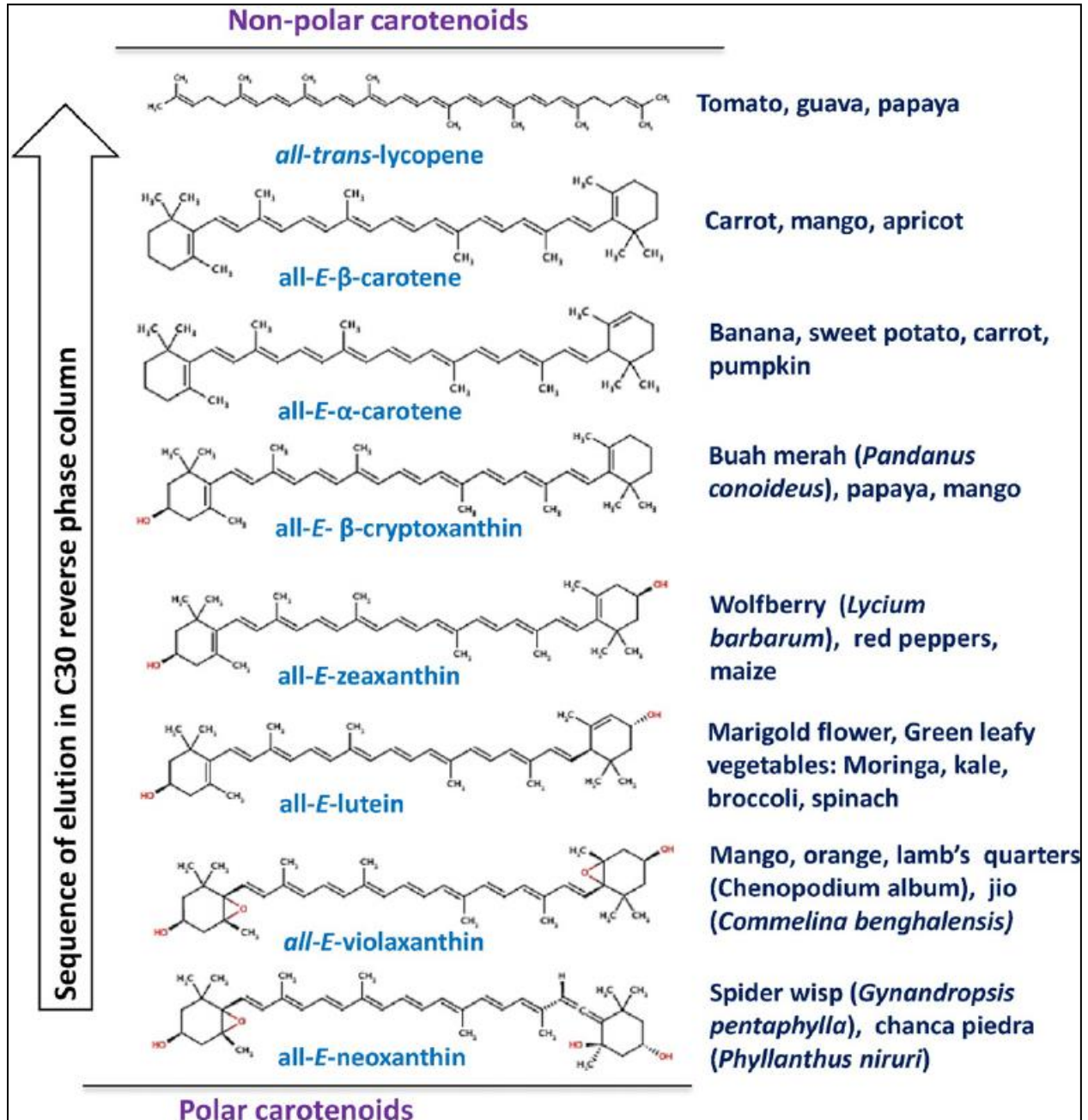
4.11 Sources: Plants, in general, contain six ubiquitous carotenoids: neoxanthin, violaxanthin, antheraxanthin, zeaxanthin, lutein and β carotene. Lutein is a yellow pigment found in fruits and vegetables and is the most abundant carotenoid in plants. Lycopene is the red pigment responsible for the color of tomatoes. Other less common carotenoids in plants include lutein epoxide (in many woody species), lactucaxanthin (found in lettuce), and alpha carotene (found in carrots). In cyanobacteria, many other carotenoids exist such as canthaxanthin, myxoxanthophyll, synechoxanthin, and echinenone. Algal phototrophs such as dinoflagellates use peridinin as a light harvesting pigment. While carotenoids can be found complexed within chlorophyll-binding proteins such as the photosynthetic reaction centers and light-harvesting complexes, they also are found within dedicated carotenoid proteins such as the orange carotenoid protein of cyanobacteria.

4.12 Classifications: Carotenoids can be classified as carotenes or xanthophylls. The carotene group comprises hydrocarbon carotenoids. Examples include α - and β -carotene and lycopene. Xanthophylls are oxidized carotenoids. Examples include lutein, violaxanthin, neoxanthin and zeaxanthin.

4.13 Chemical, physical and biological properties of Carotenoids:

Carotenoids are C₄₀ isoprenoids and are classified as tetraterpenes. Most carotenoids are yellow, but orange, green and red compounds can also be found among them. Since they are highly unsaturated (more than ten double bonds), carotenoids are colorful, easily crystallized, and prone to oxidation. Because of their oxidation sensitivity, carotenoids must be protected from light, heat, oxygen and acids during handling to prevent the samples from being destroyed. A carotenoid characteristic associated with the large number of double bonds is the occurrence of

multiple forms of isomerism, most frequently cis-trans isomerism. Of the naturally occurring isomers, however, most are in the trans form and only a small number in the cis form, since the cis isomer results in more steric hindrance between the hydrogen atoms and/or methyl groups. The cis form is usually more thermodynamically unstable and has a higher energy level than the trans form. This is because the position of electrons in the cis molecule is less advantageous.



Lecture 4

4.14 Carotenoids: Changes during processing

Being highly unsaturated, carotenoids are susceptible to isomerization and oxidation during processing and storage of foods. Isomerization of trans-carotenoids to cis-carotenoids, promoted by contact with acids, heat treatment and exposure to light, diminishes the color and the vitamin A activity of carotenoids. The major cause of carotenoid loss, however, is enzymatic and non-enzymatic oxidation, which depends on the availability of oxygen and the carotenoid structure. It is stimulated by light, heat, some metals, enzymes and peroxides and is inhibited by antioxidants. Carotenoid degradation is known to increase with the destruction of the food cellular structure, increase of surface area or porosity, length and severity of the processing conditions, storage time and temperature, transmission of light and permeability to O₂ of the packaging.

Lecture 5

Flavonoids: Sources, Functions, Classifications

4.15 Definition: Flavonoids are a class of plant and fungus secondary metabolites. Chemically, flavonoids have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C).

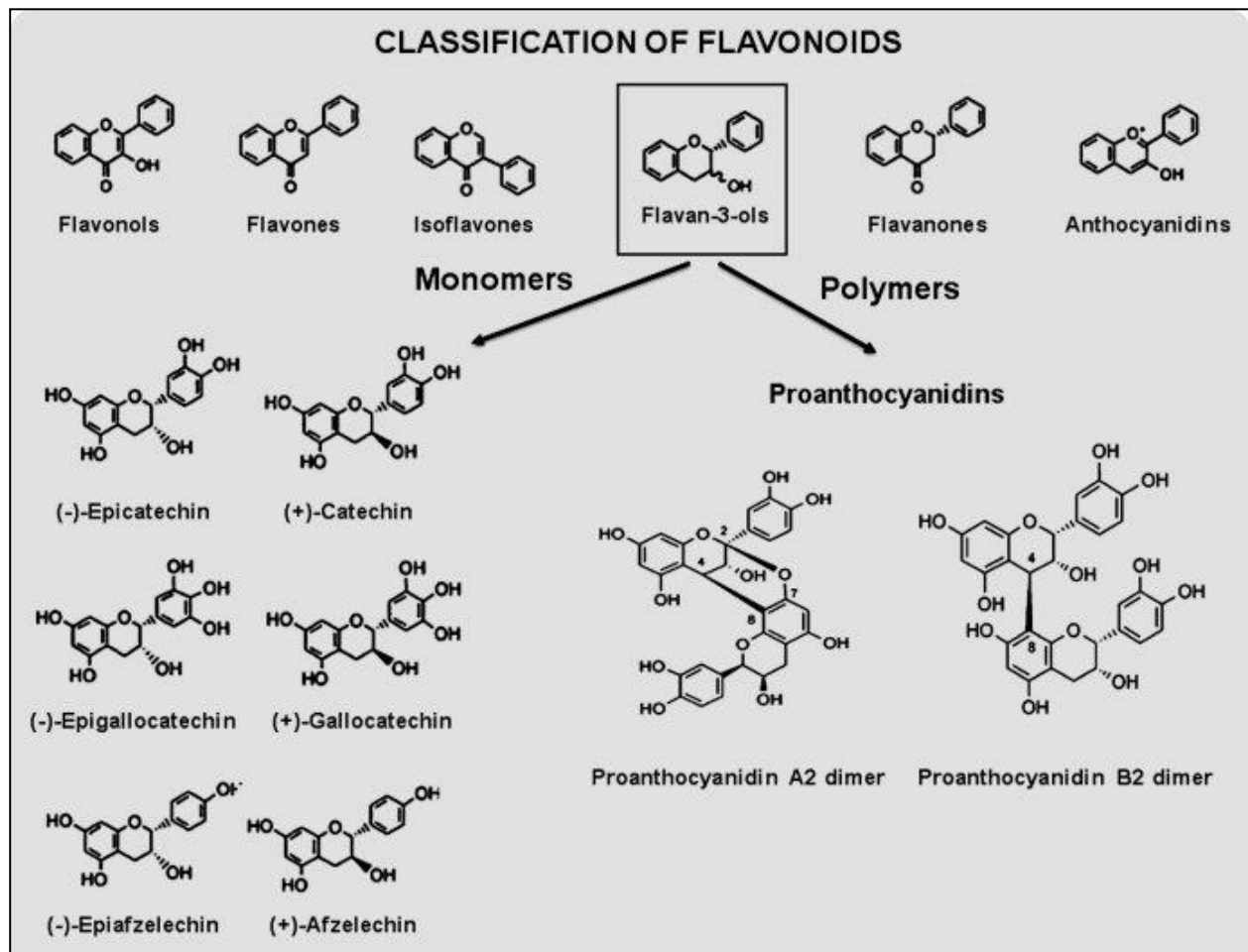
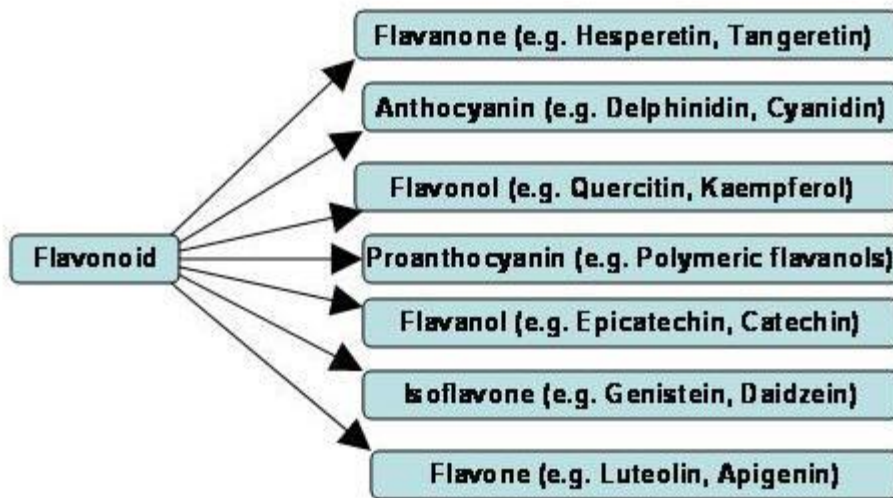
4.16 Sources: Foods with a high flavonoid content include parsley, onions, blueberries, black tea, green tea, oolong tea, bananas, all citrus fruits, red wine and cocoa.

4.17 Functions: Flavonoids are the most important plant pigments for flower coloration, producing yellow or red/blue pigmentation in petals designed to attract pollinator animals. In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. They may also act as chemical messengers, physiological regulators, and cell cycle inhibitors.

4.1 Classification: They can be classified into:

- *flavonoids* or *bioflavonoids*
- *isoflavonoids*, derived from 3-phenylchromen-4-one (3-phenyl-1,4-benzopyrone) structure
- *neoflavonoids*, derived from 4-phenylcoumarine (4-phenyl-1,2-benzopyrone) structure

The three flavonoid classes above are all ketone-containing compounds, and as such, are anthoxanthins (flavones and flavonols).



Lecture 6

4.18 Flavonoids: changes during processing

Considerable losses of flavonoids have been observed when fruits and vegetables are processed into juice because substantial amounts of the bioactive compounds are left in the discarded skin and pulp (press cake), these losses often surpassing those of heat treatment. Thermal processing has long been known to cause significant losses of anthocyanins. High temperature accelerates reactions, which would occur more slowly at ambient temperature. Whatever the processing method chosen, retention of these compounds decreases with longer processing time, higher processing temperatures and cutting or maceration of the food. Processing conditions should therefore be optimized so that bioavailability is increased, but without provoking significant degradation. Bioavailability refers to that portion of the nutrient or bioactive compound consumed that is released from the food matrix, absorbed and used by the body. In home preparation, retention of bioactive compounds decreases in the following order: microwaving > steaming > boiling. Deep-frying, prolonged cooking, combination of several preparation methods all result in substantial losses. Whatever the cooking or processing method used, retention decreases with longer cooking time, higher cooking temperature and peeling/cutting of the food. Freezing (especially quick freezing) and frozen storage generally preserve nutrients and bioactive compounds, but slow thawing may be detrimental. Traditional sun-drying, although the cheapest and most accessible means of food preservation in poor countries, causes considerable destruction of nutrients and bioactive compounds. Drying in a solar dryer, even of simple and inexpensive design, can appreciably reduce losses. Protecting the food from direct sunlight also has a positive effect.

Lecture 7

Anthocyanins: Sources, Functions, Classifications, changes during processing

4.19 Definition: Anthocyanins (literally "flower blue") are water-soluble flavonoid pigments that appear red to blue, according to pH. They occur in all tissues of higher plants, providing color in leaves, plant stem, roots, flowers, and fruits, though not always in sufficient quantities to be noticeable. Anthocyanins are most visible in the petals of flowers of many species.

4.20 Sources: Food plants rich in anthocyanins include the blueberry, raspberry, black rice and black soybean, among many others that are red, blue, purple or black. Some of the colors of autumn leaves also come from anthocyanins.

4.21 Functions: As flavonoids, anthocyanins have important roles in nature such as antioxidant, photoprotection, defense mechanisms, as well as other ecological functions (symbiosis phenomena). The relationship between the anthocyanin type (flower color) and the mechanisms of pollination, seed dispersal, and antifeedant is clearly established; as an example, delphinidin colors are common in bee-pollinated families. *Some anthocyanins act as biological control agents, such as cyanidin-3-glucoside, which inhibits larval growth in tobacco.*

It has been suggested that anthocyanins in leaves increase the amount of available light for photosynthesis and reduce the photoinhibition; this arises because anthocyanins and chlorophyll *b* absorb in the same region (520 to 530 nm).

Another important function of anthocyanins is carried out during the development of young leaves; during this period, metabolism is very active and an excess of peroxide is produced. Consequently, the production of free radicals is favored. Under these conditions, anthocyanins act as antioxidant agents by reacting with free radicals.

4.22 Classifications: Anthocyanins show high diversity in nature but all are based on a reduced number of basic anthocyanidin structure. Anthocyanins are classified by the number of sugar molecules in their structures (monosides, biosides, trisides). Color is also affected by the number of hydroxyl and methoxyl groups: if more hydroxyl groups are present, then the color goes toward a more bluish shade; if more methoxyl groups are present, then redness is increased. Six most common anthocyanidins in nature are cyanidin, pelargonidin, peonidin, delphinidin, petunidin, and malvidin.

4.23 Anthocyanins: Changes during processing: Anthocyanin stability has been shown to be affected by various physical and chemical factors, such as the chemical structure and concentration of the anthocyanins, temperature, pH, light, oxygen, presence of enzymes, proteins, metallic ions and other flavonoids and phenolics. These pigments readily degrade during processing and storage of foods, resulting in dramatic impact on color and their health-promoting properties. Suggested pathways for anthocyanin degradation involve cleavage (loss of color) or polymerization (browning). In alkaline or acid medium or in the presence of the enzyme β -glucosidase, anthocyanin is hydrolyzed, removing the sugar moiety and releasing the anthocyanidin, which is more easily degraded than the anthocyanin glycoside. Degradation commence with the opening of the middle ring followed by cleavage at this midpoint of the molecule, forming colorless products.

Lecture 8

4.24 Vitamin: food sources, functions, deficiency diseases

Vitamin	Food Source	RDA	Function	Deficiency diseases
Vitamin A (Retinol or Beta-carotene)	Liver, egg yolk, dairy products, margarine. Beta carotene (pro-vitamin A) is found in	5,000 IU	Keeps eyes healthy; develops bones; protects linings of respiratory,	Night blindness

	dark green and deep yellow fruits and vegetables.		digestive and urinary tracts; maintains healthy skin and hair. Beta carotene fights free radicals (chemicals that damage cells).	
Vitamin B ₁ (Thiamine)	Whole grains, cereals and enriched grain products; also legumes (dried beans, peas, and nuts), organ meats, lean pork and eggs.	1.1 - 1.5 mg	Promotes healthy functioning of the nerves, muscles and heart. Metabolizes carbohydrates.	Beri-beri
Vitamin B ₂ (Riboflavin)	Organ meats, enriched breads and cereals, legumes, almonds, cheese and eggs; also meat, fish and dark green vegetables.	1.3 - 1.7 mg	Metabolizes carbohydrates, fats and proteins, produces hormones; promotes eye and skin health.	Slow growth, sore eyes
Vitamin B ₃ (Niacin)	Meat, organ meats, whole grains and cereals, and legumes; also eggs, milk, green leafy vegetables and fish.	15 - 19 mg	Metabolizes carbohydrates and fats; helps functioning of digestive system; maintains health skin.	Pellagra
Vitamin B ₅ (Pantothenic Acid)	Organ meats, yeast, raw vegetables, eggs and dairy products.	None; 4 - 7 mg suggested	Produces hormones and maintains body's immune system.	Paresthesia
Vitamin B ₆ (Pyridoxine)	Whole-grain products, poultry, fish, and nuts; also meat, most fruits and vegetables, eggs and dairy products	1.6 - 2 mg	Metabolizes protein; helps produce hemoglobin; promotes functioning of digestive and nervous systems, and healthy skin.	Anemia
Vitamin B ₁₂ (Cyanocobalamin)	Primarily organ meats; also fish, lean meats, poultry, cheese, and eggs.	2 µg	Builds genetic material of cells and produces blood cells.	Megaloblastic anemia
Vitamin C (Ascorbic Acid)	Almost exclusively fruits and vegetables (especially citrus fruits, tomatoes,	100 - 200 mg	An antioxidant, fights and resists infection; heals wounds; promotes	Scurvy

	peppers, strawberries, and cantaloupe) although breast milk and organ meats contain small amounts.		growth and maintenance of bones, teeth, gums, ligaments and blood vessels.	
Vitamin D (Cholecalciferol)	For most people, sun exposure is the primary source of vitamin D. Food sources include Vitamin D-fortified milk, eggs, fish-liver oils and fatty fish such as herring, mackerel and salmon.	400 IU	Builds strong bones and teeth and maintains the nervous system.	Rickets
Vitamin E (Tocopherol)	vegetable oils, nuts, wheat germ and whole-wheat products, egg yolks and green leafy vegetables.	Women 8 mg; Men 10 mg	Protects the lungs, nervous system, skeletal muscle and the eye's retina from damage by free radicals; may reduce risk of heart disease by protecting against atherosclerosis.	Hemolysis and sterility
Vitamin H (Biotin)	Oats, organ meats, yeast and eggs (cooked); also whole-wheat products, dairy products, fish and tomatoes.	None; 30 - 200 µg suggested	Metabolizes proteins and carbohydrates; breaks down fatty acids.	Loss of appetite, depression, fatigue, dermatitis, nausea, weakness, hair loss
Vitamin K	Dark green leafy vegetables, eggs, cheese, pork and liver.	60 - 80 mg	Promotes normal blood-clotting.	Hemorrhage
Vitamin M (Folic Acid)	vegetables (especially dark-green ones), organ meats, whole-wheat products, legumes and mushrooms.	180 - 200 µg	Synthesis of protein and genetic materials; may help prevent some cancers, heart disease and stroke; when taken during pregnancy, protects against some birth defects.	Megaloblastic anemia, neurologic and psychiatric problems

4.25 Minerals: food sources, functions, deficiency diseases

Mineral	Food Source	RDA	Function	Deficiency diseases
Calcium (Ca)	Primarily in milk and dairy products; also dark-green vegetables, legumes, shellfish, fish with edible bones and tofu; also calcium-fortified orange juice.	800 - 1,200 mg	Builds bones and teeth; promotes blood clotting, contraction of muscles and nerve impulses.	Osteoporosis
Chromium (Cr)	Whole wheat and other whole grains and molasses.	None; 50 - 200 µg suggested	An essential nutrient required for normal sugar and fat metabolism; may also help prevent high cholesterol and atherosclerosis.	Fatigue, anxiety, stunted growth, poor blood glucose control, heart complications due to high cholesterol
Copper (Cu)	Organ meats, shellfish, whole-grain products, legumes and dried fruits.	None; 2 - 3 mg suggested	Builds bones, red blood cells and hemoglobin; metabolizes iron, maintains connective tissue and blood vessels; may play a role in cancer prevention.	neurologic and psychiatric disorder, heart enlargement, progressive difficulty in walking, low appetite, retarded growth
Fluoride (F)	Seafood, tea, coffee and soybeans; sodium fluoride is often added to the water supply to prevent tooth decay.	None	Promotes bone and tooth formation; prevents tooth decay.	Dental decay
Iodine (I ₂)	Saltwater fish, shellfish, sea kelp and iodized salt.	150 µg	Helps produce thyroid hormones; adequate iodine intake during	Goitre

			pregnancy is crucial to normal fetal development.	
Iron (Fe)	Iron is poorly absorbed from food. The richest sources are red meat and organ meats; other sources include whole-wheat products, shellfish, nuts and dried fruit. Many breads and cereals are enriched with iron. Vitamin C aids absorption of iron and is often added to iron supplements.	Women 15 mg; Men 10 mg	Helps produce hemoglobin and red blood cells; delivers oxygen to muscles and other body tissues; protects against effects of stress	Anemia, fatigue, irritability, weakness
Magnesium (Mg)	Legumes, whole-grain cereals, nuts and dark-green vegetables; also meat, seafood and dairy products.	Women 280 mg; Men 350 mg	Builds bones and teeth; involved in functioning of muscular and nervous systems and hear and circulatory system.	Anxiety, fatigue, insomnia, headache, irritability
Manganese (Mn)	Tea, green vegetables, legumes, oats and rice.	2 - 5 mg	Involved in reproductive processes, sex hormone formation; essential for normal brain function and bone development.	Defective ovulation, ovarian degeneration, testicular degeneration
Molybdenum (Mo)	Dairy products, legumes, whole-grain cereals and organ meats.	75 - 250 mg	Involved in enzyme activities.	Impairment in growth, diarrhea, anemia
Phosphorus (P)	Meat, fish, eggs, legumes and dairy products; also whole wheat, corn and rice.	1 gram	Builds bones and teeth.	Bad teeth and bones
Potassium (K)	Potatoes, dried fruits, bananas, legumes, raw vegetables, avocados and mushrooms; also lean meat, milk and fish.	None; 3.5 grams suggested	Helps nerves and muscles function; regulates heart's rhythm; regulates bodily fluids.	Muscle weakness, paralysis

Selenium (Se)	Whole-grain cereals, fish and shellfish, meat and dairy products.	Women 55 µg; Men 70 µg	An antioxidant, helps protect cells and tissues from damage by free radicals; may also protect against some cancers.	Cirrhosis of lever, cardiac failure, loss of appetite, nausea
Sodium (Na)	Naturally in many foods and is added to many prepared foods.	2,400 mg	Maintains body's fluid balance; important for nerve function and muscle contraction; controls heart's rhythm.	Dehydration, weakness
Zinc (Zn)	Shellfish (particularly oysters), organ meats and lean red meat, yeast, whole-grain cereals, and legumes.	Women 12 mg; Men 15 mg	Involved in growth, skin health and wound healing, development of the reproductive organs, protein metabolism and energy production.	Folate deficiency, diabetes mellitus

Question Bank

1. Multiple Choice Question (MCQ)

- i. Water helps in the metabolism process in the presence of
 - a) Amino acid, b) RNA, c) Enzyme, d) Protein
- ii. Which one of the following would give the highest energy per gram-
 - a) glucose, b) protein, c) fat, d) sucrose
- iii. Which is the urgent source of energy available for athletes?
 - a) carbohydrate, b) protein, c) fat, d) vitamin
- iv. An adult requires how many proteins daily?
 - a) 50-100 gm, b) > 100 gm, c) 30-50 gm, d) 10-20 gm
- v. Proteins are required to make which of the followings?
 - a) hormone, b) connective tissues, c) antibodies, d) all of these
- vi. The part of the cell which is essential for protein synthesis is-
 - a) golgi bodies, b) chromosome, c) chloroplast, d) ribosome
- vii. Which of the following statements about amino acids is correct?
 - a) Amino acids are classified according to the structures and properties of their side chains

b) Amino acids are uncharged at neutral pH

c) Amino acids in proteins are mainly in the D-configuration

d) Twenty four amino acids are commonly used in protein synthesis

viii. Which type of bonding is responsible for the secondary structure of proteins?

a) Disulphide bridges between cysteine residues

b) Hydrogen bonding between the C=O and N-H groups of peptide bonds

c) Peptide bonds between amino acids

d) Salt bridges between charged side chains of amino acids

ix. Which term below best defines the 'quaternary structure' of a protein?

a) The arrangement of two or more polypeptide subunits into a single functional complex

b) The folding of the polypeptide backbone in three-dimensional space

c) The interaction of amino acid side chains

d) The sequence of amino acids in a polypeptide chain

x. Hemoglobin is an example of:

a) Phosphoprotein, b) Chromoprotein, c) Prosthetic group, d) Co-factor

xi. Physical agent/s causing denaturation of proteins is:

a) UV rays, b) Urea, c) Temperature, d) Nitric acid

xii. Which of the following most accurately describes how secondary structures in proteins are stabilised?

a) Through ionic bonds operating between oppositely charged amino acid side chains

b) Through covalent bonds joining different parts of the peptide backbone

c) Through hydrogen bonds between different amino acid side chains

d) Through hydrogen bonds joining different parts of the peptide backbone

xiii. Which amino acid can form disulphide bonds?

a) Glycine, b) Proline, c) Glutamate, d) Cysteine

γ - Linolenic acid, which is one of the EFAs for humans, is an:

a) ω 2- fatty acid, b) ω 3- fatty acid, c) ω 6- fatty acid, d) ω 5- fatty acid

xv. Which of the following conformations of peptide bond, found in proteins, is more stable?

a) *cis*, b) *trans*, c) Both are equally stable, d) none of the above

xvi. A tomato gets its red color from:

a) Beta Carotene, b) Fructose, c) Lycopene, d) Limonene

xvii. Hot peppers get their heat from:

a) Acetic acid, b) Capsaicin, c) Lycopene, d) Sulfuric Acid

xviii. When you chop onions, your eyes can burn because a chemical reaction produces:

a) Acetic Acid, b) Hydrochloric Acid, c) Nitric Acid, d) Sulfuric Acid

xix. Which out of the following is not a fibrous protein?

a) carbonic anhydrase, b) keratin, c) fibrinogen, d) collagen

xx. Which of the following amino acid is a limiting amino acid in pulses?

a) leucine, b) lysine, c) methionine, d) glutamine

xxi. Choose the correct category for milk protein casein out of the following-

a) nucleoprotein, b) phosphoprotein, c) lipoprotein, d) glycoprotein

xxii. The amount of phosphovitin present in egg yolk granules is

a) 23% b) 16% c) 4% d) 75%

xxiii. The pigment present in watermelon is

a) crocetin, b) zeaxanthine, c) lycopene, d) xanthophyll

xxiv. Erythrose is a

a) Biose b) Triose c) Tetrose d) Pentose

xxv. Example of ketohexose

a) Glucose b) Fructose c) Galactose d) Arabinose

xxvi. Fatty acid profile can be determined by

a) HPLC b) IEC c) GC d) PAGE

xxvii. One of the most important protein purification method is

(a) Lane-Eynon, (b) Electrophoresis, (c) Kjeldahl, (d) All of these

xxviii. Omega- 6 rich oil is

a) Soybean oil, b) Fish liver oil, c) Sunflower oil, d) None of theses

xxix. Lard is fat from which of the following animals

a) Beef, b) Lamb, c) Chicken, d) Pig

xxx. Isoelectric pH of egg albumin is

a) 5.4, b) 4.8, c) 2.3, d) 7.0

xxx. Example of a flavor enhancer used in fast food is

- a) BHA, b) MSG, c) KCl, d) HMF

xxxii. The principle protein present in milk whey is

- a) α -lactalbumin, b) β -lactoglobulin, c) casein, d) both (a) and (b)

xxxiii. In lactose glucose and galactose are linked together by

- a) Alpha-1:4 glycosidic linkage b) Beta-1:4 glycosidic linkage
c) Alpha-1:6 glycosidic linkage d) Beta-1:4 glycosidic linkage

xxxiv. Example of heteroglycan is

- a) Hyaluronic acid, b) Cellulose, c) Starch, d) Dextra

xxxv. Example of a fat soluble pigment is

- a) Lycopene, b) Beta carotene, c) Chlorophyll, d) Both (a) & (b)

xxxvi. The flavor producing aromatic chemical in clove is

- a) MSG, b) Eugenol, c) Curcumin, d) Cinnamaldehyde

xxxvii. Naturally occurring antioxidant is

- a) Vitamin D, b) Vitamin C, c) Vitamin E, d) Both (b) & (c)

xxxviii. Loss of Mg atom from chlorophyll will produce

- a) Pheophytin, b) Chlorophyllide, c) Pheophorbide, d) None of these

xxxix. Fish liver oil consists of

- a) ETA, b) EPA, c) DHA, d) Linolenic acid

xxxx. The full form of BHT is

- a) Butylatedhydroxytoluene b) Butylated hydroxyl tampering
c) Benzene hydroxy temperature d) Bromine highly toluene

xxxxi. The yellow colour of saffron is due to the occurrence of

- a) Anthocyanin, b) Cryptoxanthin, c) Lycopene, d) crocetin

xxxxii. The provitamin of Vitamin A is

- a) Alpha Carotene, b) Beta Carotene, c) Gamma Carotene, d) All of these

xxxxiii. Starch splitting enzymes are generally

a) Lipolytic, b) Saccharolytic, c) Proteolytic, d) None of these.

xxxxiv. Hydrolysis of cellulose results the formation of disaccharide as

a) Maltose, b) Lactose, c) Cellobiose, d) Sucrose

xxxxv. The co-enzyme of Vitamin B₆ is

a) TPN, b) TPP, c) PALPO, d) None of these

xxxxvi. Zein is an example of

a) Wheat protein, b) Milk protein, c) Egg protein, d) Corn protein

xxxxvii. The meaning of the Greek word “Chroma” of “Chromatography” is

a) to estimate, b) colour, c) band, d) None of these

xxxxviii. Example of water in oil emulsion is

a) Mayonnaise, b) Margarine, c) Butter, d) Both (b) & (c)

xxxxix. Peptide bonds are found in

a) Uric acid, (b) Insulin, (c) Urea, (d) None of these

L. The principle of gel filtration lies on

a) Size and shape, b) Solubility, c) Ionization, d) All of these

Li. Quercetin is a type of

a) flavones, b) carotenoids, c) chlorophyll, d) None of these

Lii The approximate water activity of foods at which the bacterial growth starts is

a) 0.4, b) 0.6, c) 0.8, d) 1

Liii. Keratin is a

a) Fibrous protein, b) Globular protein, c) Conjugated protein, d) Derived protein

Liv. Zein is deficient of the following two essential aminoacids

a) Leucine & Isoleucine b) Lysine & Tryptophan

c) Threonine & Methionine d) Arginine & Histidine

Lv. Myosin is a

a) Cereal protein, b) Fish protein, c) Meat protein, d) None of these

Short answer questions (SQ)

2. Classify the naturally occurring pigments. Describe how the pigments changed during curing of meat. Why the colour of carrot is yellow? (1+3+1=5)
3. Differentiate between: (any two) (2.5x2=5)
- a) Stacking Gel and Running Gel
 - b) Paper Chromatography and Thin Layer Chromatography
 - c) Fibrous protein and Globular Protein
 - d) Rod Gel Electrophoresis and Slab Gel Electrophoresis
4. Name the different techniques used for identification and separation of proteins. What is peptide linkage? Write down the principle of gel filtration. (2+1+2=5)
5. Why is the green colour of vegetable lost during thermal processing? Name the pigments present in saffron. What is the major mineral present in chlorophyll molecule? (3+1+1=5)
6. Compare between reducing and non-reducing sugar. (5)
7. Write a short note on glycogen. (5)
8. What do you mean by EAA? Give one example each of sulphur containing and aromatic ring containing amino acid. Name the principle protein present in corn. (2+2+1=5)
9. What is pro-vitamin? Discuss the stability of Vitamin C during processing and storage. (1+4=5)
10. Write a short note on trans fats. (5)
11. What is water activity? How water activity affects the microbial spoilage of foods? (1+4=5)
12. What do you understand by IMF? Give some examples. State the relationship between water activity and equilibrium relative humidity. (2+1+2=5)
13. Explain the occurrence of anthocyanin in plant cells. Briefly discuss the processing loss of anthocyanin. (2+3=5)
14. Write down two chemical properties of proteins. Classify proteins on the basis of solubility with examples. (2+3=5)

15. Briefly discuss the changes during processing & storage stability of water soluble vitamin. Mention one vitamin considered as free radical quencher? (4+1=5)

16. Define iodine value and peroxide value. What are their Chemical significance? (2+3=5)

17. Write the differences between amylose and amylopectin. (5)

18. What are porphyrins? How plant porphyrin derivatives undergo changes on storage and on processing? (1+4=5)

19. Explain why carrots show loss of colour on drying. Give an example of vitamin acts as an antioxidant. (4+1=5)

20. Write the functions of cholesterol? Discuss its positive and negative effects on health. (5)

21. Why proteins are called amphoteric in nature? Write down the structure of the following Amino Acid. a) Methionine, b) Tyrosine, c) Isoleucine (2+3=5)

22. Write short note on egg protein. (5)

Long Answer Type Questions (LQ)

23. Milk protein casein contains high amount of calcium- Justify. Explain the principle involved during preparation of cheese. Name the antimicrobial proteins present in egg. (2+3+2=7)
(b) What are flavonoid compounds? Mention the different types of flavonoids related compounds present in food materials. Briefly discuss the processing loss of anthocyanin. (1+3+4=8)

24. Write the different oxidation reactions of glucose reduction reaction of fructose. . What changes occur after gelatinization? What is dextrinisation of starch? Explain retrogradation of starch with example. (7+2+3+3=15)

25. (a) What is α -tocopherol? Comment on its stability and degradation in food materials. Why is the colour of carrot yellow? Carrots show loss of colour on drying- explain. Name the pigment responsible for red colour of tomato. (1+2+2+4+1=10)

(b) Why is the green colour of vegetable lost during thermal processing? Name the major mineral present in chlorophyll molecule. (4+1=5)

26. a) What is meant by denaturation of protein? Mention the factors which cause the denaturation. Give two examples of essential amino acid with structure. (1+3+2=6)

b) Explain the two major reasons why casein is resistant to heat destruction compare to most other food proteins. (4)

c) Write a short note on properties of protein. (5)

27. Classify the fatty acids with examples. Explain omega-3 and omega-6 fatty acids. Why they are called essential fatty acids? Explain hydrolysis of fats showing reactions of different steps. (5+4+2+4=15)

28. Briefly describe moisture absorption isotherm of food material and state the relationship of three zones of moisture with it. How food's stability with respect to relative reaction rate is related to water activity? (7+8=15)

29. Write a short note on Electrophoresis. State the principle involved in Gel filtration process in context to protein purification. Give one example each of basic, acidic and neutral amino acid. (6+6+3=15)

30. Explain Beer-Lambert law. With schematic diagram state the principle of photoelectric colorimeter. Distinguish between Spectrophotometric and Colorimetric method- which one is more accurate method for detection of the components having very close absorbance value? (4+8+3=15)

31. Explain the physiological functions of carbohydrate. What do you understand by optical activity of monosaccharide? Explain homoglycan and heteroglycan with examples. (8+3+4=15)

32. Describe lecithin. How it is act like an emulsifier? What do you understand by reversion of fats and oils. How it differs from rancidity? What is the side effect of partial hydrogenation of fats. (3+3+3+3+3=15)

33. Discuss the change of color occurs during heat processing of chlorophyll and also explain its storage stability. How flavor constituent of spices can be influenced by heat treatment? What are the color components of peach, orange, cowpea and egg yolk? State the function of MSG in food. (5+4+4+2=15)

34. Describe the different types of hydrolysis of starch, amylose and amylopectin. Classify gums from their sources and give examples. What do you understand by available and unavailable carbohydrates? (7+4+4=15)

35. Classify the lipids with examples. Explain the physical properties of fats. (6+9=15)

36. What is globular protein? What kind of changes occur in meat during curing process? What is actomyosin? How β -carotene adds to nutritional value of food materials? Briefly explain the role of Calcium and Sodium in our body. Why are casein micelles stable to precipitation by heat and acid? (2+4+1+2+3+3=15)

37. What do you understand by glass transition? Classify Tg. How the different changes in states of food material are related with Tg- explain with diagram. What is the importance of determination of moisture content of food materials? (2+2+5+6=15)

38. a) Name one fat soluble vitamin and explain its role in human nutrition. Give one example of flavor intensifier. Name two methods used for estimation of protein content of food. (1+2+1+1=5)

b) Frozen red raspberries show excellent retention of colour than in canned berries- explain. Name the pigments present in apricot and papaya. (3+2=5)

c) Classify protein according to the solubility. Give example each of phospho-protein and metallo-protein. (3+2=5)

39. Write short note on: (any five)

(5x3)

- Zwitterions,
- Peptide linkages
- EAA
- Principle involved in Paper Chromatography
- Classification of Chromatography
- Classification of Wheat protein
- Egg protein
- Meat Protein

40. Write down the principle of Electrophoresis. Explain the separation method of casein by Polyacrylamide gel electrophoresis (PAGE). State the principle involved in SDS-gel electrophoresis. (4+8+3=15)