UNIT I

1.1 Carbohydrates

Carbohydrates are most abundant biomolecules in nature, which consists of C, H, O and having general formula $–C_n(H_2O)_n$. These are defined as the polyhydroxy aldehydes (or) ketones which produce them on hydrolysis. A polyhydroxy compound indicates more than one hydroxyl group and a carbonyl group. They are also known as saccharides it means sugar. Your body converts these vital food ingredients into glucose, which provides you with the energy you need to perform.

Eg: Glucose, Lactose, Maltose etc.

Classification

Carbohydrates are classified into 4 types.

Monosaccharides: water soluble crystalline compounds.
Eg: fruits, vegetables, honey, nuts.

Disaccharides: major source of energy in animals.
Eg: lactulose, maltulose, chitobiose.

Oligosaccharides: low molecular weight polymers of monosaccharides.
Eg: sugars, milk.

Polysaccharides: high molecular weight polymers of monosaccharides.
Eg: rice, potatoes, corn, wheat.

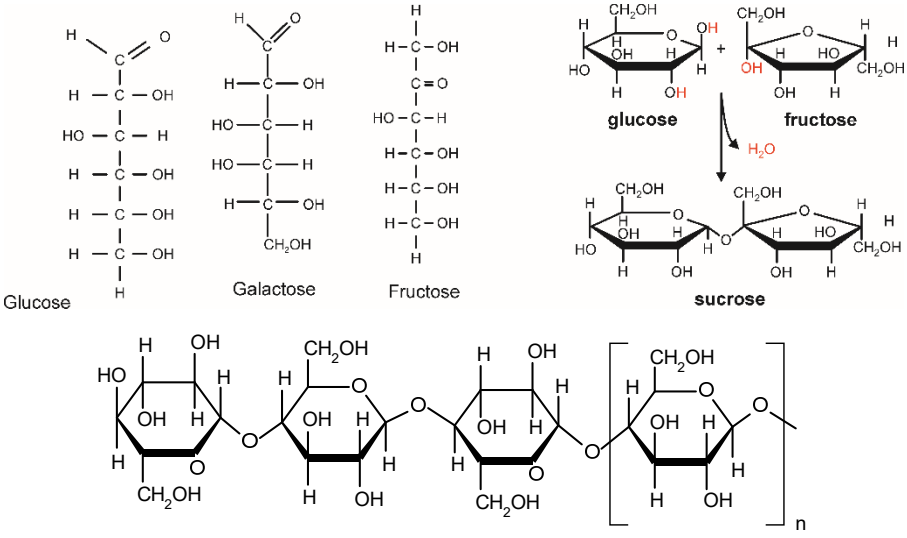

Fig. 1.1 General structure of carbohydrates.

MONOSACCHARIDES:

They cannot be hydrolyzed into smaller carbs, making them the most basic type. In monosaccharides, the term "mono" denotes the existence of a single sugar unit.

Eg: Glucose, Fructose and Galactose.

DISACCHARIDES:

A disaccharide is created when two simple sugar molecules are joined to one another. Two monosaccharide units are released when they undergo a dehydration process. The term "glycosidic bond" refers to the covalent bond that develops between two molecules of sugar.

Eg: Sucrose, Lactose, Maltose.

OLIGOSACCHARIDES:

Three to ten molecules of the same or different monosaccharides are produced when oligosaccharides undergo hydrolysis. Monosaccharides are joined via glycosidic linkages. Tetra-, penta-, and trisaccharides, among others are the several classifications for oligosaccharides.

They are divided into two categories.

i. **N-linked oligosaccharides:** These are oligosaccharides that have a beta link to the amine nitrogen on the side chain, which binds them to aspargine.
ii. **Oligosaccharides that are O-linked:** These are oligosaccharides that bind to the threonine of the side chain's hydroxyl group. such as stachyose and raffinose.

POLYSACCHARIDES

A chain of more than ten carbohydrates connected by the formation of glycosidic bonds is called a polysaccharide. They're again classified into 2 types.

i. Homopolysaccharides contains the same type of monosaccharides.

   Eg: Starch, Cellulose

ii. Heteropolysaccharides contains the different type of monosaccharides.

   Eg: Pectin, Hemicellulose.

1.1.1 Properties of Food Carbohydrates

Hygroscopicity: Hygroscopic means that it attracts water. When sugar is exposed to air, it absorbs moisture from the air and is best kept in an airtight container. When sugar and sugar-based confections are exposed to air, they have a tendency to absorb moisture and become sticky.

Solubility: Both mono and oligosaccharides are well soluble in water. As the sugar combines, as it frequently does in sugar powder or granulates, solubility diminishes. Fructose, sucrose, glucose, maltose, and lactose are the sugars that are listed in decreasing order of solubility. This characteristic is crucial for obtaining a specific product when using a sugar mixture.

Crystallization: Sugar's capacity to dissolve and re-form crystals. When sugar dissolves in a liquid and the liquid evaporates, the sugar solution becomes concentrated. Sugar crystallizes as a solution cool. The resulting sugar crystals are highly sought-after in sugar-coated foods like sugar-coated almonds and other items like ices and candies. The type of crystallizing medium, the amount of sugar in the preparation (the higher the concentration, the faster the rate of crystallization), the temperature at which crystallization occurs, the stirring of the sugar preparation, and the addition of additional ingredients like butter, ghee, egg, etc. are some of the variables that affect it.

Gelatinization: Starch granules expand by swelling and finally burst when heated in a solution of water and starch, absorbing liquid and thickening the mixture. If sufficient starch is added, a gel will form when it cools. It usually occurs at 88 to 90 degrees Celsius. Common thickening agents include maize flour, plain wheat flour, and others. The properties of the gel are influenced by two things. These include the food's preparation technique and the ratio of amylose to amylopectin. Partial or total gelatinization is possible.

i. When the starch granules are dextrinized before being boiled in water, this is known as partial gelatinization (gummy). For instance, sheera and upma.

ii. **Total Gelatinization:** After thoroughly mixing starch and water and cooling, total gelatinization will occur.

Gelation: It is a technique for solidification that primarily involves freezing to create gel or solidify any gelation solution. Gelation is accomplished with gelatin. When frozen, the gelatin liquid continuously thickens into a thick sugar solution. No specific setup point exists. Some foods form gel when cooled, while others may form gel when heated. The type of gelation occurred determines whether the gel is thermo-irreversible or thermo-reversible. For instance, boiling egg whites and cooling gelatin to cause gelation

Viscosity: The thickness or resistance to flow of a fluid is known as its viscosity. A liquid's viscosity increases with its thickness. Such as honey, syrup, etc.

Foaming: It is created when tiny gas bubbles are scattered across a liquid. For example, when whisking liquid egg white, air bubbles are integrated into the resulting egg white foam.

Colloidal property: When two substances disperse but do not mix to form a solution, colloids are created. The state of two chemicals combined together determines the type of colloidal system. The majority of colloids are stable, but over time, a temperature increase or physical force may cause the two materials to split. They will occasionally become unstable when frozen or heated, particularly if they contain an emulsion of water and fat.

1.1.2 General Methods of Analysis of Food Carbohydrates

SAMPLE PREPARATION:

Due to complex nature of foods, analysis of carbohydrates is normally preceded by sample purification. This process is used for removal of possible interference. It involves extraction of mono & oligosaccharides and removal of interfering compounds by clarification with lead acetate and clarification with ion exchange resins.

Method of analysis involves the following: Chemical, Enzymatic and Physical methods.

CHEMICAL METHODS:

Since the majority of them are reducing sugars, these techniques are utilized to identify mono and oligosaccharides. It is depending on how reducing sugars react with chemical reagents to produce colored or precipitate complex. The final stages of these methods involve gravimetric analysis, titration or spectrophotometric determination at a specific wavelength.

Lane-Eynon Method:

This method is an example of a titration method for determining how much reducing sugar is in a sample. It is based upon the reduction of sugars in a copper sulfate solution, followed by a reaction with alkaline tartrate (or by treatment with the Soxhlet solution: an equal volume mixture of alkaline tartrate solution and copper sulfate solution). To eliminate nearly all of the copper in the Soxhlet solution, the sample solution can be added by burette once the approximate carbohydrate concentration of the sample is established. Methylene blue is then added to this mixture after it has been boiled for a very specific amount of time—two minutes. The original sample solution is then used to titrate this colored solution. again, within a specific time period (3 min) until decolouration of the indicator. This method is a time-specific assay that is dependent upon the heating time, temperature, and reactant concentration; in the hands of the untrained it can lead to inaccurate results. The technique is frequently employed to identify the reducing sugars in honey and other syrups with high reducing sugar content.

Munson and Walker Method:

The Munson and Walker Method is a gravimetric method for determining the concentration of reducing sugars. It involves the oxidation of the carbohydrates under carefully regulated circumstances, with heat, excess cupric sulfate, and alkaline tartrate presence. To maintain copper in solution as copper hydroxide (Cu), basic conditions are needed. Heat causes water to evaporate and copper oxide changes into cuprous oxide, which precipitates as the carbohydrates oxidize and become detectable.

i. The electrode's weight growth is correlated with the decreasing sugar content when copper oxide is electrolytically deposited onto platinum electrodes after being dissolved in nitric acid.
ii. By using a titration method wherein nitric acid dissolves cuprous oxide It is oxidized to cupric nitrate, potassium iodide is added, and the iodide is oxidized to iodine. A starch indicator is then used to titrate the iodine with thiosulphate.
iii. Potassium permanganate in which cuprous oxide is reacted with ferric sulfate and the ferric ion is reduced to the ferrous ion (+3 to +2); the After that, permanganate is used to titrate the ferrous ion, changing its color due to permanganate (+3, pink +2, colourless).

Nelson Somogyi Method:

This method to measure reducing sugars is a modification of the Munson and Walker and Lane-Eynon methods, which are applicable for samples that contain low concentrations of carbohydrate. Ammonium molybdate and sodium arsenate are reacted in sulfuric acid to create the arsenomolybdate reagent, which is used in the synthesis of cuprous oxide with it. When cupric is oxidized to cuprous by reducing sugars and the arseno-molybdate complex is also reduced, a very stable solution with a deep blue hue is produced (because of the reduced arsenomolybdate). This solution's absorbance is measured at either 500 or 520 nm. This method requires preparation of a standard curve.

Alkaline Ferric Cyanide Method:

This method, first introduced in 1962, is based on the principle that carbohydrates in a basic solution (pH > 10.5) can reduce ferricyanide to ferrocyanide. Ferrocyanide can then react with ferric ions to produce Prussian blue, which can be read spectrophotometrically at 700 nm. The resulting blue colour is quite stable, and the reaction obeys Beer's law. However, standards must be employed to obtain accurate results.

Phenol Sulphuric Acid Method:

This is an illustration of a colorimetric technique for determining the total amount of sugar in a sample. Because sulfuric acid is used, this method makes it possible to quantify both reducing and non-reducing carbohydrates. The test's foundation is the response of phenol and carbohydrates with a strong acid presence, which generates heat, and then heating to 25–30°C for 20 minutes. Furfural and hydroxymethyl furfural are produced when the carbohydrates are dehydrated in highly acidic environments. After that, phenol condenses these compounds. The entire reaction produces a yellow-orange color that may be detected by spectrophotometry at 480 nm for pentose and 490 nm for hexose.

Anthrone Method:

This is an illustration of a colorimetric technique for figuring out how much sugar is present in a sample overall. When carbohydrates combine with 9,10-dihydro-9-oxoanthracene (anthrone) in an acidic environment (such as sulfuric acid), a blue-green hue is produced. Following 15 minutes of heating in boiling water, the reaction mixture is left to cool in the dark (20–30 minutes for color creation) before the absorbance at 620 nm is measured. Since strong sulfuric acid is added as part of the process, both reducing and nonreducing carbohydrates (hexoses) are identified.

Other Methods:

- **i.** Reducing sugars react with 2,3,5-triphenyl tetrazolium bromide or chloride above pH 12.5 to produce a coloured complex, the triphenyl formazon (pink-violet-blue, measured at 485 nm). The final colour of the reaction mixture depends on the carbohydrate.
- **ii.** Reducing sugars also react with 3,5-dinitrosalicylate under alkaline conditions to produce red-brown colours that can be measured spectrophotometrically.
- **iii.** Carbohydrates react with resorcinol under strongly acidic conditions to give coloured complexes. All hexoses react under these conditions; however, the colour yield with ketoses is much greater than that for aldoses. Therefore, this method can be used to provide analytical information on keto sugars.
- **iv.** Carbohydrates react with orcinol in strongly acidic conditions to give coloured complexes. This method has been used to determine pentoses in the presence of hexoses because the colour response for a hexose is approximately 5 percent of that for a pentose. The colour formed is quite stable, and the absorbance can be measured at 670 nm.

ENZYMATIC METHODS:

Enzymes are proteins with catalytic activity due to their power of specific activation. Because these compounds are proteins, they are susceptible to denaturation (loss of catalytic activity) by pH, temperature, and other environmental factors. In most cases, enzymes that are used for analytical purposes have a maximum temperature range of 2060°C and pH values from 4 to 10. Due to their high specificity and sensitivity, enzyme assays are ideal for the analysis of food carbohydrates. Assays may be performed on an aliquot of the food itself without partial or total separation of the carbohydrates prior to analysis. These enzymatic reactions are usually conducted at temperatures close to room temperature, at neutral pH, and in minutes (typically <20 min), thus minimizing changes in the compounds during analysis.

- **i.** D-Glucose/ D-Fructose/ D-Sorbitol Method
- **ii.** Lactose /D-Glucose Method
- **iii.** Maltose/Sucrose/D-Glucose Method
- **iv.** Raffinose method

PHYSICAL METHODS

The carbohydrate content of foods has been ascertained using a variety of physical techniques. These techniques depend on the fact that a food's physicochemical characteristics change when its carbohydrate content changes. The following techniques are frequently employed: specific gravity, refractive index, and polarimetry.

Specific Gravity:

Specific gravity is defined as the ratio of the density of a substance to the density of a reference substance.

$$S = \frac{Da}{Db} = \frac{\text{weight of Xml of substance}}{\text{weight of Xml water}}$$

The specific gravity measurements can be measured by a variety of methods like Pycometer, Westphal balance, Hydrometer. These can be analysed for invert sugars, sucrose, maltose and maltotriose.

Refractive Index:

When electromagnetic radiation travels through different media, it can change direction; it is either bent or refracted. The ratio of the sine of the angle of incidence to the sine of the angle of refraction is termed the index of refraction (n). Refractive index varies with concentration, temperature, and the wavelength of light. A carbohydrate solution's refractive index increases with increasing concentration and can be used to estimate the amount of

carbohydrate present. The refractive index is also temperature and wavelength dependent and measurements are usually made at a specific wave length (589.3nm) and temperature (20^{o}C). This method is simple and quick to perform and can be carried out with simple hand-held instruments. It is utilized to determine concentrations of sugar in honey, syrups, jams and molasses in industry.

Polarimetry:

The majority of substances with an asymmetric carbon atom can rotate polarized light's plane of polarization. A polarimeter measures the rotatory power exerted by a compound in solution on plane polarized light. The polarimeter consists of a source of monochromatic radiation; a polarizer for converting the light waves in the beam of monochromatic light into plane polarized light (light that vibrates in only one plane). Polarimetry utilizes the carbohydrates' optical activity. It means that the plane polarized light can be rotated through an angle by carbohydrates and that is dependent on the nature of sample, concentration of the sample, wavelength of the light and temperature.

1.1.3 Changes in Food Carbohydrates during Processing, Digestion, Absorption and Metabolism of Carbohydrates

Changes in Food Carbohydrates during Processing:

Leaching-induced loss of carbohydrates:

i. **Carbohydrates with low molecular weight:** Micronutrients and When fruits and vegetables undergo wet heat treatment, such as blanching, boiling, and canning, a large amount of low molecular weight carbohydrates, or mono- and disaccharides, are lost into the processing water. For instance, 25% and 30% of the carbohydrates in carrots and swedes are lost during the blanching process.
ii. **Dietary fiber:** Boiling, blanching, and canning green peas, carrots, brussels sprouts and green beans have not been shown to release dietary fiber into the processing water. However, boiling reduced the amount of dietary fiber (mostly insoluble) in swedes by 40%.

Variations low molecular weight carbohydrates:

a) **Resistant oligosaccharides production:** Enzyme technology is a rapidly developing technique for producing resistant oligosaccharides. The two most widely used forms of resistant oligosaccharides are galacto-oligosaccharides formed from lactose and fructo-

oligosaccharides formed from sucrose. Alternatively, inulin can be hydrolyzed to create fructo oligosaccharides.

b) **Maillard reactions:** During food processing and storage, reducing sugars and amino groups undergo non-enzymatic browning processes. The most widespread of these temperature-dependent reactions occurs at intermediate water activity. They are crucial for nutrition because they can reduce the bioavailability of amino acids, particularly lysine, which lowers the nutritional value of protein.

Effects of heat on starch:

a) **Gelatinization:** Starch, when heated along with water at high temperature, due to excessive heat molecules break to form gelatin.

b) **Retrogradation:** Retrogradation is the term for the recrystallization, which may make the starch less digestible. A long-term phenomenon that happens gradually when starchy meals are stored is the retrogradation of the amylopectin component. On the other hand, amylose re-associates faster. Retrograded amylopectin loses its crystallinity after being reheated to about 70°C, but retrograded amylose requires temperatures above 145°C to lose its crystallinity. This temperature is far higher than the range at which starchy foods are processed. This suggests that once created, retrograded amylose will maintain its crystallinity after the food has been reheated.

c) **Par-boiling:** This method involves heating and drying the rice kernels as a pre-treatment. By enabling reduced the stickiness of the rice by allowing leached amylose to retrograde and/or create inclusion complexes with polar lipids on the kernel surface. The rice's ultimate cooking qualities are also impacted by parboiling.

Texturization of starch:

In pasta products, gluten forms a visco-elastic network that surrounds the starch granules, which restricts swelling and leaching during boiling. Pasta extrusion is known to result in products where the starch is slowly digested and absorbed. This not only restricts swelling, but possibly also results in a more gradual release of the starch substrate for enzymatic digestion. Pasta is now generally acknowledged as a low glycemic index food suitable in the diabetic diet.

Dietary fiber:

a) **Milling and peeling:** The outer fiber-rich layers are eliminated when cereal grains are ground into refined flours, which lowers the amount of total dietary fiber. The primary cause of this decrease is a decline in insoluble fiber. Whole-grain and refined flours have varied amounts of nutritional fiber. Barley, oats, sorghum and rice refined flours are

primarily composed of glucans, whereas maize, rye, and wheat refined flours are primarily composed of arabinoxylans. Cellulose is present in significant levels in all whole-grain flours.

b) **Heat-treatment:** Heat-treatment procedures can have a variety of effects on dietary fiber. Weak connections between polysaccharide chains disintegrate when the temperature rises. Additionally, the polysaccharides in the dietary fiber may break their glycosidic bonds. From an analytical, functional, and nutritional perspective, these modifications are significant. Even under mild conditions, the solubility of the dietary fiber rises when wheat flour is extruded and cooked. It appears that the amount of water used in the procedure affects the solubilization; the less water used, the more the fiber is dissolved.

c) **Hydration qualities (water-binding, swelling, and water-holding capacity):** Grinding pea hull fibers reduces their water-binding and swelling capacity while marginally increasing their water-holding capacity.

DIGESTION:

It happens mostly in the gut and momentarily in the mouth as follows:

- **Mouth:** Salivary amylase (ptyalin) breaks down alpha-1,4-glycosidic linkages in starch during mastication and forms disaccharides (dextrin, maltose).
- **Stomach:** The enzyme salivary amylase is inactivated in stomach due to high acidity; hence the digestion (or) degradation of starch is stopped.
- **Small intestine:** Two different categories of enzymes are acted on starch, one from pancreas another from succus entericus and convert different polysaccharides into disaccharides and monosaccharides.
- **Final products:** monosaccharides like glucose, fructose, galactose.

Table 1.1 Carbohydrate digestion.

Area	Juice	Enzyme	Substrate	End product
Mouth	Saliva	Amylase (ptyalin)	Poly saccharides	Di saccharides (dextrin,maltose)
Stomach	Gastric juice	Gastric amylase(inactive)	No action	
Small intestine	Pancreatic juice	P. amylase (amylopsin)	Poly saccharides	Di saccharides (dextrin, maltose)
	Succus entericus	Sucrase	Sucrose	Glucose, fructose
		Maltase	Maltose	Glucose
		Lactase	Lactose	Glucose, galactose
		Dextrinase	Dextinose	Glucose
		Trehalase	Trehalose	Glucose

ABSORPTION:

It occurs mostly in duodenum and the small intestine's upper jejunum. The end results of digesting are absorbed as follows.

Glucose: Glucose transported from sodium co-transport from the small intestine into the epithelial cells in the mucous membrane. Glucose enters the portal vein by enhanced diffusion from epithelial cells. Since the entrance of Na+ into the intestinal lumen is an active process that requires energy, the intestinal Na+ gradient serves as the immediate energy source for glucose transport, which is indirectly provided by ATP.

Galactose: It is similar as that of glucose.

Fructose is simply carried by carrier-mediated enhanced diffusion. Certain fructose molecules undergo conversion to glucose, which is then absorbed as shown in Figure 1.2.

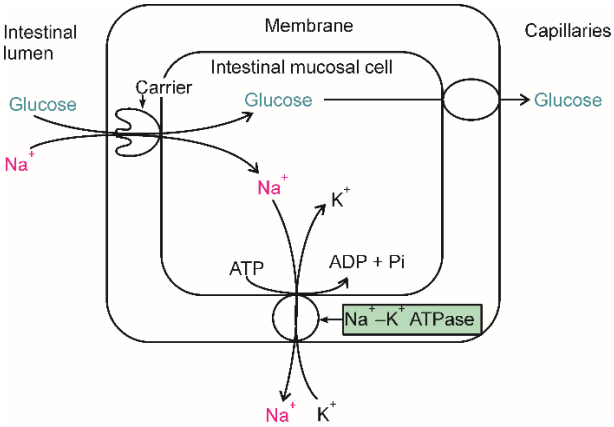

Fig.1.2 Absorption of carbohydrates.

METABOLISM:

Following digestion, food must be absorbed and used by the body through an oxidation process called catabolism, in which all food is burned slowly to release energy. Rich energy phosphate is stored as a portion of the released energy used by the tissues for physiological functions bond known as anabolism. Carbohydrate metabolism occurs as shown in Figure 1.3.

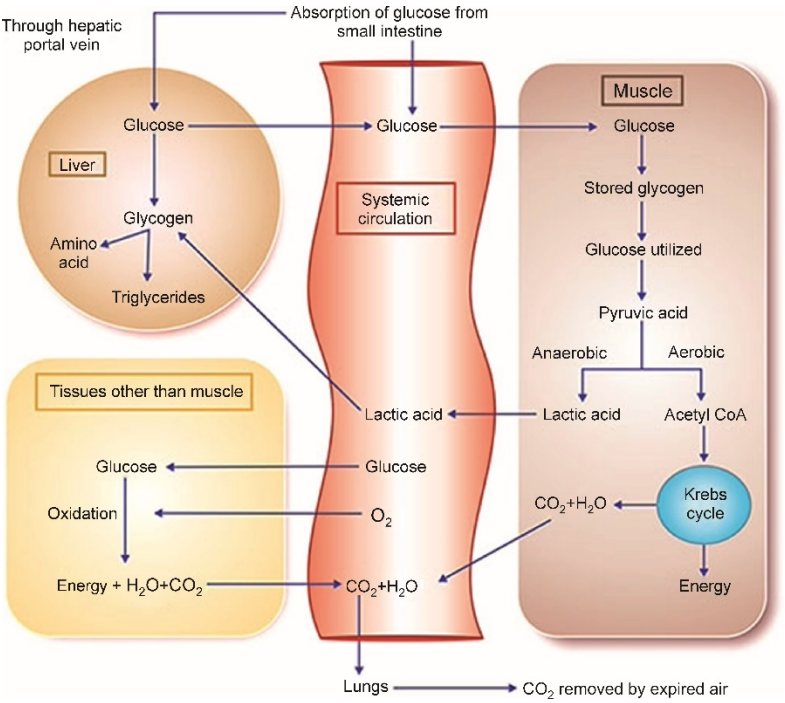

Fig. 1.3 Metabolism of carbohydrates.

1.1.4 Dietary Fiber

Dietary fiber is the part of food generated from plants that digestive enzymes are unable to break down completely. It is the edible parts of plants that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. These promote beneficial physiological effects including laxation, blood cholesterol attenuation, blood glucose attenuation. Components include cell wall polysaccharides, noncell wall polysaccharides & lignin's.

Cell wall polysaccharides:

- **Cellulose:** β-D-glucopyranosyl units form the linear polymer known as cellulose. Ten thousand or more glucosyl units may be present in some compounds.
- **Hemicellulose:** The sole resemblance among the diverse collection of polysaccharides known as hemicellulose is their interaction with cellulose in plant cell walls. The main-chain structures of hemicelluloses are usually composed of units of D-xylose, Dmannose, and D-galactose; side chains or branch units of L-arabinose, D-galactose, and uronic acids are also common.

- **Pectins:** A linear chain of 1,4-linked a-D-galacto pyranosyl uronic acid units is the primary characteristic of all commercial pectins. Neutral sugar unit segments may branch out, perhaps accompanied by other polysaccharides. Methyl ester is a common type of carboxylic acid groups found in D-galacturonic acid units.
- **Non cell wall polysaccharides:** Mucilages, gums, alignates.

Lignins:

It is a non-carbohydrate, three-dimensional, water-insoluble polymer and a major component of the cell walls of higher land plants. Lignin may be covalently linked to hemicellulose.

There are two categories of dietary fiber:

a) **Soluble fiber, which includes pectin, glucose, and maltose:** In the colon, it readily ferments to produce gases and physiologically active byproducts including short-chain fatty acids that are created by gut bacteria in the colon, and it dissolves in water. It is viscous, sometimes referred to as pre-biotic fiber, and it slows down emptying of stomach, which might give people a prolonged feeling of fullness. Soluble fibre attracts water and turns to gel during digestion. Some soluble fibres may help to prevent cardio-vascular diseases. Soluble fibre may help to lower the cholesterol level and helps to regulate blood sugar levels. Soluble fibre is found in oat bran, barley, nuts, seeds, beans, peas, some fruits and vegetables.

b) **Insoluble fibre (Cellulose, Hemi-cellulose, and Lignin):** This gives bulky results and is inert to digestive enzymes in the upper gastrointestinal tract. It also does not dissolve in water. The colon can ferment resistant starches and other types of insoluble fiber. As bulking fibers pass through the digestive tract, they absorb water, which eases defection, preventing constipation. Insoluble fibre is found in wheat bran, vegetables, wholegrain.

1.1.5 Crude Fibre

It is a cellulose material obtained as a residue in the chemical analysis of vegetable 12 substances. It is a measurement of the amount of lignin, pentosans, indigestible cellulose, and other similar substances found in food. It is what's left over after plant materials are extracted using a solvent and then digested using diluted acid and alkali. Despite having little nutritional value, these ingredients give the intestinal tract the weight it needs for healthy peristaltic motion. Crude fiber is made up of Cellulose (60 to 80%) and Lignin (4 to 6%).

1.1.6 Application of Food Carbohydrates

- Simple syrup (sucrose) preparation.
- As binders and diluents in tablet manufacturing.
- To coat pills coated with sugar.
- To prepare food for newborns.
- To make a sterile intravenous solution.
- In medications that treat diarrhoea.
- As antacids, diuretics, or laxatives.
- As agents that emulsify.
- As nutritional media for tissue culture and bacteria.
- When making plasters and surgical dressings.
- Certain carbohydrates, such as dextran, glucosamine, and ascorbic acid, have a variety of medicinal applications.

1.2 Protein

1.2.1 Introduction

Proteins are large and complex organic compounds which play very critical roles in the body. It is involved in the composition, operation, and control of numerous organs &body tissues. These large, intricate biological molecules are L-alpha-amino acid polymers.

Protein and amino acid chemistry and classification

CHEMISTRY

Amino acids are the organic compounds which contain two functional groups i.e., amino group (NH2) and carboxylic acids group (COOH). The amino group is basic in nature and carboxylic acid group is acidic in nature.

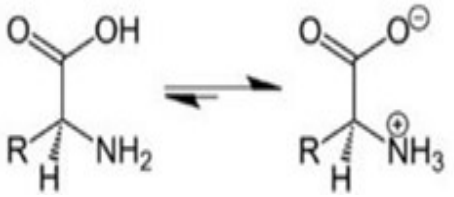

Fig. 1.4 General structure & zwitter ion of amino acid.

Amino acids exists as zwitter ion in both acidic pH (i.e.,< 4) and basic pH (i.e.,> 9) and between pH 4 to 8 it exists in neutral form. Depending on the number of amino acids and their sequence different types of proteins are present in nature. About 300 types of amino acids are present in nature but only 20 amino acids are present in structure of proteins. The R group varies

for every one of the 20 amino acids that make up proteins. In the biological system, they are primarily found in ionic form.

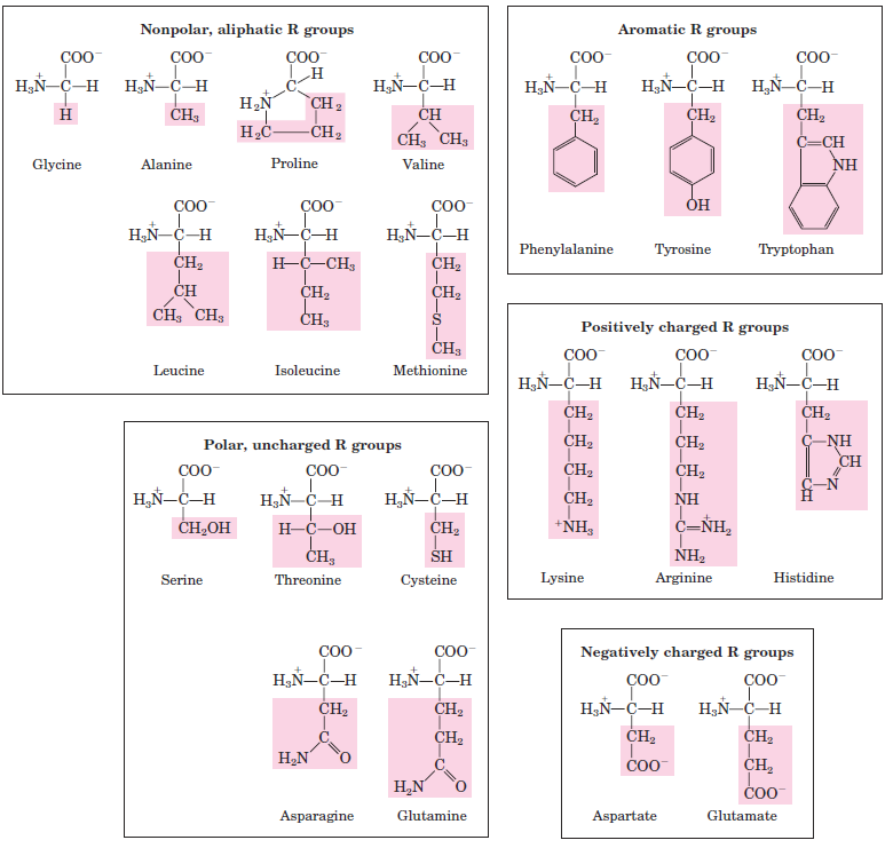

Fig. 1.5 Structure of amino acids based on R groups.

Amino acid optical isomers: A carbon atom is asymmetric and exhibits optical isomerism if it is joined to four distinct groups. All amino acids have four different groups (R, H, COOH, and NH2) that are housed by alpha carbons, with the exception of glycine. Thus, there are optical isomers of every amino acid except glycine. There are two kinds of the optical isomers: L and D.

CLASSIFICATION OF AMINO ACIDS

There are numerous ways to categorize amino acids according to their chemical nature, polarity, nutritional needs, and metabolic fate.

Structure-based classification of amino acids

They are separated into many groups according on their structure and chemical makeup.

i. **Aliphatic side chains containing Amino acids:** These are mono amino mono carboxylic acids. This group comprises of the simplest amino acids. For instance, the branching chain amino acids valine, leucine, isoleucine, glycine, and alanine.

ii. **Amino acid with Hydroxyl group:**

Eg. Tyrosine, Serine and Threonine.

iii. **Sulfur containing amino acids:**

Eg. Thioether group - Methionine

Sulfhydryl group - Cysteine

Dimer formed by condensation of two molecules of cysteine - Cystine

iv. **Amino acids and their amides:** These are the dicarboxylic mono amino acids.

Eg. Glutamic acid- Glutamine

Aspartic acid- Asparagine

v. **Basic amino acids-** These are dibasic mono carboxylic acids. They are highly basic in nature.

Eg. Lysine, Arginine, Histidine

vi. **Aromatic amino acids:** These amino acids contain aromatic groups in their structure.

Eg. Phenyl alanine, Tryptophan and Tyrosine

Polarity based classification of Amino acid

Based on polarity these are classified into 4 types.

i. **Non- polar amino acids:** These are called to as hydrophobic (water hating). They have no charge on the R group. Eg. Alanine, Proline, Valine, Leucine, Isoleucine, Methionine, Phenyl alanine, and Tryptophan

ii. **Polar amino acids with no charge on R group:** The R group don't carry any charge. They have groups like hydroxyl, sulfhydryl, and amide groups that help proteins form hydrogen bonds in protein structure. For instance, glutamine, asparagine, glycine, serine, threonine, cysteine, and tyrosine.

iii. Polar amino acids that include a positive R-group include histidine, arginine, and lysine

iii. **Polar amino acids with an R-group that is positive:** For instance, glutamic acid and aspartic acid

***Amino acid classification based on Nutrition*:**

All amino acid need not be taken in diet. These are the amino acids which

i. **Essential (or) indispensable amino acid:** cannot be synthesized by the body. Therefore, need to be supplied through diet. They are required for proper growth and maintenance of individual.

   Eg. Histidine, Isoleucine, Lysine, Leucine, Arginine, Threonine, Tryptophan, Methionine, Valine, Phenyl alanine. (Arginine and Histidine are called semi-essential amino acids since they are produced by adults but not by children, so their name)

ii. **Non-essential (or dispersible) amino acids:** These are the amino acids that the body synthesizes and do not require dietary intake.

   Eg. proline, glutamate, glutamine, tyrosine, glycine, alanine, serine, cysteine, aspartate, and asparagine.

***Classification of Amino acids based on metabolic fate*:**

Amino acid carbon skeletons can be used as building blocks to synthesize fat, glucose, or both

i. **Glycogenic amino acids:** These amino acids act as building blocks to create glucose or glycogen. For instance, Alanine, Aspartate, Glycine, Methionine

ii. **Ketogenic amino acids:** These amino acids serve as precursors for the synthesis of fat. Eg. Leucine and Lysine

iii. **Ketogenic and Glycogenic amino acids:** These amino acids are building blocks for the production of fat and glucose. For instance, tyrosine, tryptophan, phenyl alanine, and isoleucine

1.2.2 Physico-chemical Properties of Protein

The physicochemical qualities of the amino acids vary, which in turn dictate the features of proteins.

Physical characteristics

a. **Solubility:** The majority of them are insoluble in organic solvents and soluble in water.

b. **Melting Point:** Proteins generally melt at temperatures higher than 200°C.

c. **Taste:** Bitter (arginine, isoleucine), sweet (valine, leucine, glycine, alanine). The food industry uses monosodium glutamate (MSG) ajinamoto as a flavoring agent.

d. **Optical properties:** Because of the asymmetric carbon atom, all compounds—apart from glycine—display optical isomers. A second

asymmetric carbon atom can be found in certain amino acids such as isoleucine and threonine.

e. **Ampholytic nature:** They contain both acidic and basic groups so they can either donate a proton or accept a proton.

f. **Isoelectric pH:** Every amino acid carries both positive and negative charges and exists as a zwitter ion at a specific pH. We refer to this pH as isoelectric pH where it contains no net charge. Thus, the molecule is electrically neutral.

Chemical properties:

The chemical properties are attributed because amino and carboxylic acid groups are present.

Reactions brought on by the COOH group:

i. They combine with alcohols to generate esters (COOR) and salts (COONa).

ii. **Amines are produced by decarboxylation:** This is an important reaction in the biological system. It produces some important amines. Eg. Histidine produces histamine, Tyramine produces Tyrosine, Glutamate produces GABA.

iii. **Reaction with ammonia:** It produces amides. Eg. Glutamic acid + NH_3→Glutamate, Aspartic acid + NH_3→Asparagine.

The NH_2 group causes the following reactions:

i. The amino groups act as bases and react with acids (like HCl) to produce salts.

ii. **Reaction with ninhydrin:** A purple, blue, or pink complex is created when the alpha amino acids and ninhydrin interact.

Amino acid + Ninhydrin → Ketoacid + NH_3 + CO_2 + Hydrindantin

Hydrindantin + NH3 + Ninhydrin → Ruhemann purple

It is useful for quantitatively determining proteins and amino acids. Hydroxyproline and proline gives the color of yellow.

iii. **Color reactions of amino acids:** Amino acids can be identified by specific color reactions. Ninhydrin reaction (alpha amino acids), Biuret reaction (two peptide links), and Xanthoprotic reaction (benzene ring of aromatic aminoacids)

iv. **Transamination:** To create a new amino acid, an amino group is transferred from an amino acid to a keto acid. This process is known as transamination. This process is crucial to the metabolism of amino acids.

v. **Deamination by oxidation:** Oxidative deamination is the liberation of free ammonia from the amino group of amino acids coupled with oxidation.

1.2.3 Structure of Proteins

Proteins are L-alpha-amino acid polymers. The protein structure is complex with four levels of organization.

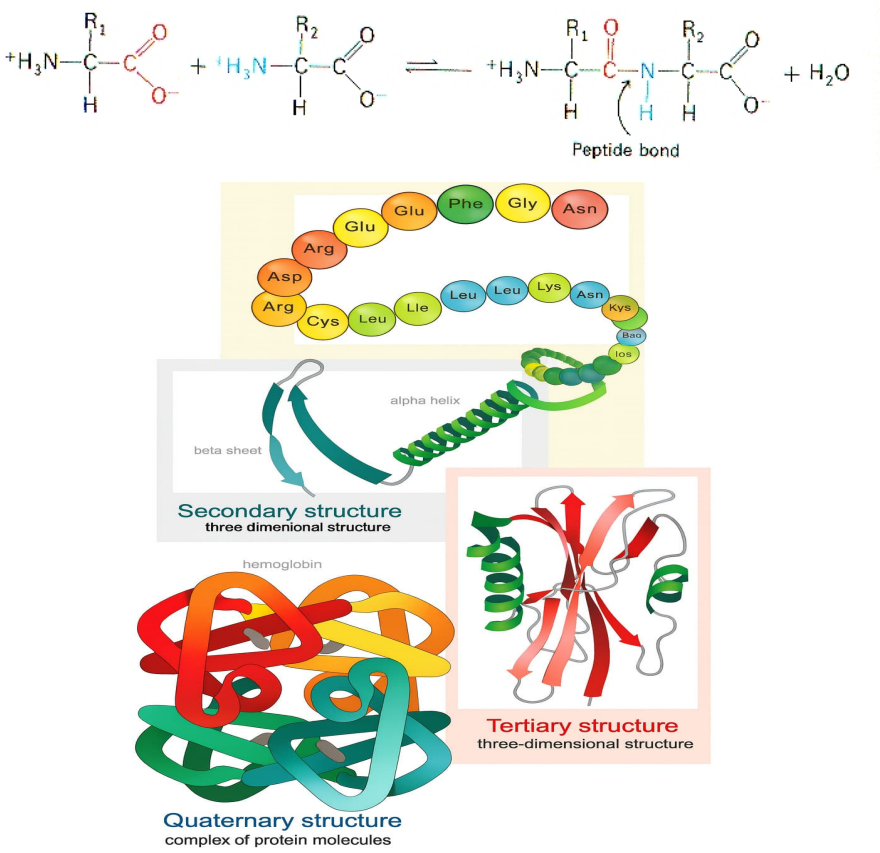

Fig. 1.6 Structure of proteins.

Primary structure: It is the backbone of proteins made up of the linear arrangement of amino acids. Its function is mostly due to the primary structure.

***Peptide bond*:** When an of carboxyl group of amino acid combines with the amino group of another amino acid, a peptide bond is formed. The free carboxyl end (C-terminal residue) is written at the right of the peptide chains, whereas the free amino acid (N-terminal residue) is written at the left. From the N-terminal end to the C-terminal end, the amino acid sequence is read.

Secondary structure: The conformation of polypeptide chain by twisting or folding is secondary structure. They locate close to each other. Two types of secondary Structures, alpha helix and beta pleated sheets.

***Alpha helix*:** It is the most common spiral structure. It had a rigid arrangement. With amino acids extending outward from the center axis, the helix is a densely packed coiled structure. Numerous hydrogen bonds stabilize the alpha helix. It is created by joining the oxygen atom of one peptide with the hydrogen atom of another. The helix has 3.6 amino acids per turn and has a 0.54 nm travel distance. Each amino acid is separated by 0.15 nm spacing. Compared to the left-hand helix, the right-hand helix is more stable. Alpha helices structure is disrupted by some amino acids, such as proline.

Beta pleated sheets: They are made up of two or more fully expanded peptide chain segments. Hydrogen bonds are created between adjacent segments of peptide chains. R-Groups are directed to both outside and inside of the sheet. Beta sheet can be parallel, anti-parallel or mixed.

***Tertiary structure*:** It is how proteins are arranged in three dimensions. Because the hydrophilic groups are on the protein molecule's surface and the hydrophobic side chains are retained inside, the structure is compact. This gives stability to the protein. Besides the hydrogen bonds, disulfide bonds, ionic interactions and vanderwaals forces also contribute to this structure.

Quaternary structure: Some of the proteins are composed of two or more polypeptide chains referred to as subunits. The spatial arrangement of these subunits is known as quaternary structure. The monomeric subunits are held together by non- covalent bonds namely hydrogen bonds, ionic bonds and hydrophobic interactions.

Protein characteristics:

a. Proteins are soluble and form colloidal solutions in water due to their large size.

b. **Molecular weight:** It depends on the no. of amino acids. The molecular weight of approximately 110 daltons which is influenced by each amino acid.

Amino acids- 40 to 4000, molecular weight- 4000 to 4, 40,000. Eg. Insulin- 5700, Myoglobin- 17,000

c. **Shape:** Oval (Albumin), fibrous (Fibrinogen), and globular (insulin).

d. **Isoelectric pH:** When at this pH, they are electrically neutral with minimum solubility, maximum precipitability and least buffering capacity.

e. **Acidic and basic proteins:** proteins in which the ratio of total no of Lysine, Arginine and Histidine to the total no of Aspartate and

Glutamate is greater than 1 are considered as basic proteins and in which the ratio is less than 1, are considered as acidic proteins.

f. **Protein Precipitation:** Dehydration or neutralization of polar groups can precipitate proteins. precipitation of proteins occurs through Salting out, By addition of salts of heavy metals, By addition of anionic(or) alkaloid reagents, By addition of organic solvents.

g. **Protein color reactions:** Proteins exhibit color reactions that can be used to determine the type of amino acids they contain.

Classification of Proteins:

Proteins are classified mainly based on their function, chemical composition, solubility characteristics, and nutritional significance.

Classification based on function

i. Structural proteins: collagen in bone, keratin in hair and nails
ii. Catalytic proteins or Enzymes: Pepsin and hexokinase
iii. Transport proteins: serum albumin and haemoglobin
iv. Actin and myosin are examples of contractile proteins.
v. Storage proteins include glutelin and ovaalbumin.
vi. Insulin, Growth hormone are hormone proteins.
vii. Nucleoproteins are genetic proteins
viii. Immunoglobulins and snake venoms are examples of defense proteins
ix. Acetylcholine is a receptor protein.

Based on chemical nature and solubility

i. Simple proteins are made up only of residues from amino acids.
ii. The prosthetic group, also referred to as the conjugating group, is a non-protein component that is present in *conjugated proteins* in addition to the amino acids.
iii. **Derived proteins:** These are simple and conjugated proteins' denatured or broken-down byproducts.

i. **Simple proteins:**

a. **Globular:** Oval or spherical, water-soluble, and digestible.

- Water dissolves albumins. Eg. Ovalbumin (egg), lactalbumin (milk), and serum albumin
- **Globulins:** Dissolve in diluted and neutral salt solutions. Vitelline (egg yolk) and serum globulins, are examples

- **Glutelins:** Found primarily in plants, they dissolve in diluted acids and alkalies. For instance, oryzenin (rice) and glutelin (wheat)
- **Prolamines:** A 70% alcohol solution will dissolve them. For instance, Zein (maize) and Gliadin (wheat)
- **Histones:** are soluble in water and diluted acids, they are strongly basic for instance, thymus
- **Globins:** These proteins are not basic. Eg. Androglobin, Myoglobin, cytoglobin, hemoglobin etc.
- **Protamines:** They are soluble in ammonium hydroxide, have a strong basicity, and resemble histones but are smaller. Sperm proteins, for instance.
- **Lectins are proteins** that bind carbohydrates and are created when cells and proteins interact. Eg. Concanavalin A, Agglutinin

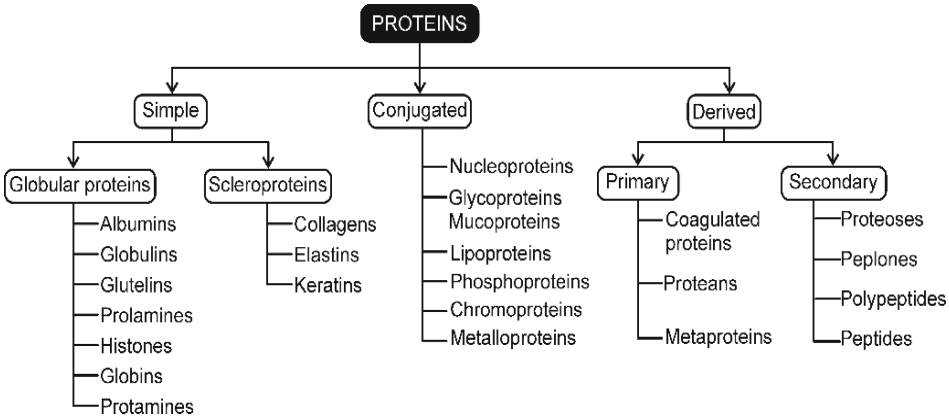

Fig. 1.7 Classification of proteins.

b. Fibrous: Fiber like in shape, insoluble in water and resistant to digestion.

- **Collagens:** connective tissue proteins with insufficient tryptophan. Gelatin is created when it is cooked with water or diluted acids.
- **Elastin:** Present in arteries and tendons.
- **Keratins:** Found in the exoskeletal system. such as horns, claws, and hair

ii. Conjugated proteins:

- **Nucleoproteins:** The prosthetic group is nucleic acid, which can be either DNA or RNA. For instance, nucleoprotamines and nucleohistones
- **Glycoproteins:** Prosthetic group is Carbohydrate, which is less than 4%, if it is more than 4%, it is called mucoprotein.

  Eg. Mucin (saliva), Ovomucoid (egg white)
- **Lipoproteins:** Protein as the prosthetic group. Eg. Serum lipoprotein
- **Phosphoprotein:** The prosthetic group is phosphoric acid.

  Examples of include casein (found in milk) and vitellin (found in egg yolk).
- **Chromoproteins:** The prosthetic group is naturally colored. For example, cytochromes and hemoglobin
- **Metalloproteins:** Metal ions including Fe, Co, Zn, Cu, Mg, and others are found in metalloproteins.

iii. Proteins derived from

a. Primary derived proteins are proteins that have been denatured, coagulated, or initially hydrolyzed.

- Proteins that have been denatured by heat, acids, alkalis, etc. are known as coagulated proteins. For instance, coagulated albumin (egg white) and cooked proteins
- Proteans are insoluble in water and are created when enzymes, diluted acids, alkalis, etc., hydrolyze proteins. For instance, fibrinogen forms fibrin.
- Metaproteins are byproducts of the hydrolysis of proteins that are produced by treating them with slightly more potent acids and alkalis. For instance, acid and alkali metaproteins

b. Secondary derived proteins: Protein hydrolysis produces these successive hydrolytic compounds. For instance, peptides, peptones, polypeptides, and proteases

Nutritional classification

- Complete proteins are those that include all 10 essential amino acids in the proper ratios needed by the body to support healthy growth. For example, milk casein and egg albumin

- Proteins that are partially incomplete can support moderate development because they partially lack one or more amino acids. Rice and wheat proteins, for instance
- **Proteins that are incomplete:** One or more necessary amino acids are totally absent from these proteins. As a result, they do not encourage growth at all. As an example, tryptophan is deficient in gelatin and tryptophan and lysine are deficient in zein.

1.2.4 General methods of Analysis of Proteins and Amino Acids

Kjeldahl method:

This process uses sulfuric acid and catalysts to break down and digest proteins and other organic food ingredients in a sample. Ammonium sulfate is produced from the total of organic nitrogen. After being neutralized with alkali, the digest is distilled into a solution of boric acid. Standardized acid, which is transformed into nitrogen in the sample, upon titrating the formed borate anions. The analysis's outcome indicates the food's crude protein content.

Procedure: This method includes 3 steps:

i. **Digestion:** Sample (10-15mg) reacts with sulphuric acid (helps in digestion of protein) copper sulphate (catalyst) and sodium sulphate (increase boiling point and increase rate of reaction) in digestion flask produces ammonium sulphate, water and carbon dioxide. Here the ammonia is not liberated as it is combined and in the form of ammonium sulphate.

$$\text{Sample} + H_2SO_4 + CuSO_4 \rightarrow (NH_4)_2SO_4 + H_2O + CO_2.$$

ii. **Digestion/Neutralization:** In this step the ammonium sulphate reacts with NaOH /Alkali to liberate free ammonia.

$$2NaOH + (NH_4)2SO_4 \rightarrow 2NH_4 + 2H_2O + Na_2SO_4 + CO_2$$

The free ammonia is transferred to another flask which already has low pH and contains

Boric acid.

$$NH_3 + H_3BO_3 \rightarrow NH_4 + H_3BO_3$$

iii. **Determination of Ammonia/Titration:** The liberated ammonium ions are titrated with HCl.

$$H_2BO_3 + H^+ \rightarrow H_3BO_3$$

Moles of HCl = Moles of NH_3 = Moles of N in sample

$$\% N = V \times 0.0014 \times 100 / W$$

$$\% \text{ Protein} = \% N \times 6.25.$$

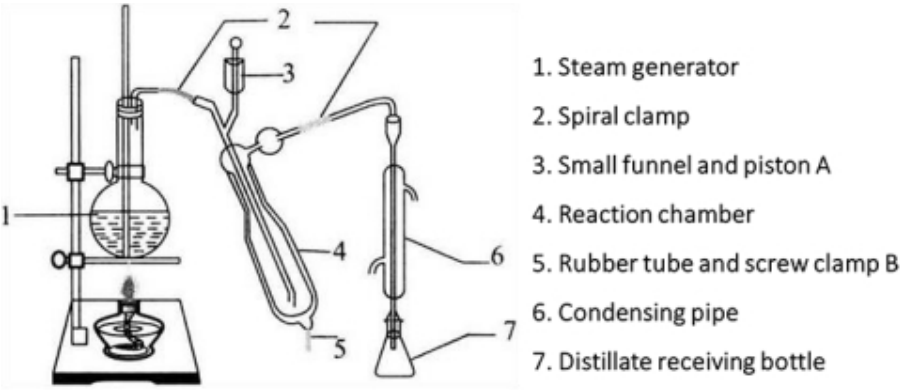

Fig. 1.8 Apparatus for Kjeldhal method.

Benefits: Suitable for all kinds of foods, accurate and cost-effective. Protein is measured in minute (mg) amounts using the modified Kjeldahl technique.

Drawbacks: measures not just the nitrogen in proteins but also the total nitrogen. Corrosive reagents, Poorer precision than biuret method, Time consuming.

Biuret Method:

In alkaline conditions, cupric ions combine with peptide bonds (substances having at least two peptide bonds, such as biuret, big peptides, and all proteins) to give a violet-purplish color. At 540 nm, the color's absorbance is measured. The sample's protein concentration is directly correlated with the color intensity (absorbance).

Procedure: One milliliter of protein solution (1–10 mg protein/ml) is combined with five milliliters of biuret reagent. To stabilize the cupric ion in the alkaline solution, the reagent consists of potassium sodium tartrate, copper sulphate, and NaOH. The absorbance at 540 nm is then measured against a reagent blank after the reaction mix has been let to remain at room temperature for 15 or 30 minutes. If the reaction mixture is unclear, filtration or centrifugation must be performed before reading absorbance. Bovine serum albumin (BSA) is used to create a standard concentration vs absorbance curve.

Benefits: Cheaper than the Kjeldahl method; quick (less than 30 minutes); and the simplest approach to protein analysis. Compared to Lowry, ultraviolet (UV) absorption, or turbidimetric approaches (discussed below), color discrepancies are less common. Food proteins are the only compounds that disrupt the biuret reaction. Does not pick up nitrogen from sources that are not proteins or peptides.

Drawbacks: Needs at least 2-4 mg of protein for the experiment; less sensitive than the Lowry method. Bile pigments may contribute to absorbance if they are present. The reaction is hampered by high ammonium salt concentrations. varied proteins have varied colors; gelatin has a pinkish-purple hue. If there are significant amounts of fat or carbohydrates in the final solution, opalescence may happen. Color must be standardized against a known protein (like BSA) or against the Kjeldahl nitrogen method; this is not an absolute approach.

The Lowry Method

Tyrosine and tryptophan residues in the proteins reduce the Folin Ciocalteau phenol reagent (phosphomolybdic-phosphotungstic acid) in the Lowry method, which also incorporates the biuret reaction. The bluish hue created is measured at 500 nm (low sensitivity for high protein concentration) or 750 nm (high sensitivity for low protein concentration).

Procedure: The proteins that are to be analyzed are diluted to the proper concentration (20–100 μg). After cooling, Na K Tartrate-Na_2CO_3 solution is added, and the mixture is incubated for 10 minutes at room temperature. After cooling, the $CuSO_4$-K Na Tartrate-NaOH solution is added, and it is then allowed to sit at room temperature for ten minutes. After adding freshly made Folin reagent, the reaction mixture is combined and incubated for ten minutes at 50 degrees Celsius. At 650 nm, absorbance is measured. A meticulously built BSA standard curve is used to estimate the unknown protein's concentration.

Advantages: Very sensitive (50–100 times more sensitive than biuret method, 10–20 times more sensitive than 280-nm UV absorption method, similar sensitivity as Nesslerization; however, more convenient than Nesslerization. Less affected by turbidity of the sample. More specific than most other methods. Relatively simple; can be done in 1–1.5 h.

A disadvantage is that color and protein content are not exactly proportionate. Hexosamines, monosaccharides, phosphate buffers, lipids, and sucrose all impede the process to differing degrees. High levels of sulfhydryl compounds, ammonium sulfate, and reducing sugars disrupt the process.

Dye-Binding procedure:

There are two kinds of it:

Anionic dye binding method

Bradford dye binding method

Anionic dye binding method

The sample -containing protein is combined with a known excess of anionic dye in a buffered solution using the anionic dye binding method. The color is

bound by proteins to create an insoluble compound. Following reaction equilibration and the elimination of the insoluble complex by centrifugation or filtration, the amount of unbound soluble dye is determined.

Excess dye + Protein → Protein−dye insoluble complex + unbound

Procedure: The sample is added to an excess dye solution with a known concentration after being finely powdered (60 mesh or less). The material is filtered or centrifuged to get rid of insoluble materials after being shaken forcefully to balance the dye binding processes. A dye standard curve is used to determine the dye content and measure the absorbance of the unbound dye solution in the filter or supernatant. Plotting the unbound dye concentration against the food's total nitrogen (as assessed by the Kjeldahl method) over a broad range of protein composition yields a straight calibration curve. The calibration curve or a regression equation derived using the least squares method can be used to estimate the protein content of an unknown sample of the same food type.

Benefits: Quick (15 minutes or less), affordable, and reasonably reliable for determining the amount of protein in food products. Since the dye does not bind changed, unavailable lysine, it may be used to estimate changes in the amount of lysine that is available in cereal products throughout processing. The available lysine content indicates the protein nutritional value of the cereal products because lysine is the limiting amino acid in them. Absence of corrosive agents. Does not quantify nitrogen that is not a protein. greater accuracy than the Kjeldahl technique.

Drawbacks: The insensitivity and the need for milligram amounts of protein are the drawbacks. The basic amino acid composition of proteins varies, which affects how well they bind to dyes. A calibration curve is therefore necessary for a particular food commodity. Because it binds to the N terminal of amino acids, it is not appropriate for hydrolyzed proteins. Certain nonprotein substances (like starch) or proteins (like calcium or phosphate) can bind dye, leading to mistakes in the end product. By employing a correctly buffered reagent that contains oxalic acid, the issue with calcium and heavy metal ions can be resolved.

Bradford Dye-Binding Method:

The color of Coomassie Brilliant Blue G-250 shifts from reddish to bluish when it binds to protein, and its maximum absorption wavelength moves from 465 to 595 nm. The sample's protein concentration is directly correlated with the change in absorbance at 595 nm.

Procedure: 85% phosphoric acid is used to acidify Coomassie Brilliant Blue G-250 after it has been dissolved in 95% ethanol. The Bradford reagent is combined with samples that contain proteins (1–100 μg/ml) and standard

BSA solutions. A reagent blank is used to compare the absorbance at 595 nm. The BSA standard curve is used to measure the protein concentration in the sample.

Benefits: Quick; it takes two minutes to complete the reaction. Reproducible. More sensitive than the Lowry method by several folds. No interference from cations like K+, Na+, and Mg+2, polyphenols, carbohydrates like sucrose, or ammonium sulfate. Estimates proteins or peptides with molecular masses of at least 4000 molecules.

Drawbacks: Both ionic and non-ionic detergents, including sodium dodecyl sulphate and Triton X-100, interfere with it. However, with the proper controls, mistakes caused by trace levels (0.1%) of these detergents can be fixed. Quartz cuvettes can bind to the protein-dye complex. Glass or plastic cuvettes must be used by the analyst. Distinct protein kinds have distinct colors. Care must be used when choosing the standard protein.

The Bicinchoninic Acid Method:

Under alkaline conditions, proteins and peptides convert cupric ions to cuprous ions, which is conceptually comparable to the biuret reaction. A purplish complex is formed when the cuprous ion and the apple greenish bicinchoninic acid (BCA) reagent combine. For a broad range of protein concentrations, from micrograms to 2 mg/ml, the color at 562 nm is almost linearly proportional to the protein content.

Procedure: Combine the protein solution with the BCA reagent, which includes copper sulphate, sodium carbonate, BCA sodium salt, and NaOH at a pH of 11. Incubate for 30 minutes at 37°C, 2 hours at room temperature, or 30 minutes at 60°C. The required level of sensitivity determines the temperature to be used. The color reaction increases with temperature. Compare the solution to a reagent blank at 562 nm. Use BSA to create a standard curve.

Advantages: Sensitivity is comparable to that of the Lowry method; sensitivity of the micro- BCA method (0.5–10 μg) is better than that of the Lowry method. One-step mixing is easier than in the Lowry method. The reagent is more stable than for the Lowry reagent. Medium concentrations of denaturing reagents don't get involved.

Drawbacks: The color does not hold up over time. The time for reading absorbance must be carefully managed by the analyst. Any substance that has the ability to convert Cu+2 to Cu+ will produce color. Compared to the Lowry process, reducing sugars interfere more. High ammonium sulfate concentrations also cause problems. Protein color fluctuations are comparable to those found in the Lowry technique.

280 nm ultraviolet Method of Absorption:

Because of their tryptophan and tyrosine residues, proteins exhibit high absorption in the ultraviolet (UV) 280 nm area. Beer's rule can be used to determine the concentration of proteins based on the absorbance at 280 nm because the amount of tryptophan and tyrosine in proteins from different food sources is rather constant.

Procedure: Proteins are dissolved in alkali or buffer. A reagent blank is used to measure the protein solution's absorbance at 280 nm. The formula A = abc (A = absorbance; a = absorptivity; b = cell or cuvette path length; c = concentration) is used to determine protein concentration.

Advantages: Fast and somewhat sensitive; requires at least 100 μg of protein at 280 nm; several times more sensitive than the biuret technique. Ammonium sulfate and other buffer salts don't interfere. Non-destructive; frequently employed in post-column protein detection; samples can be used for other studies following protein determination.

Drawbacks: At 280 nm, nucleic acids also absorb. Pure protein and nucleic acids have absorption 280 nm/260 nm ratios of 1.75 and 0.5, respectively. If the ratio of 280 nm to 260 nm absorption is known, the absorption of nucleic acids at 280 nm can be corrected. A technique based on the absorption differential between 235 and 280 nm can also be used to repair nucleic acids. Proteins from different food sources include varying amounts of aromatic amino acids. The solution ought to be colorless and transparent. Particles in the solution will cause turbidity, which will artificially raise absorbance. This approach requires a relatively pure system.

Dumas (Nitrogen Combustion) Method:

The combustion method was introduced in 1831 by Jean-Baptiste Dumas. Samples are combusted at high temperatures (700–1000°C) with a flow of pure oxygen. All carbon in the sample is converted to carbon dioxide during the flash combustion. Nitrogen-containing components produced include N2 and nitrogen oxides. The nitrogen oxides are reduced to nitrogen in a copper reduction column at a high temperature (600°C). Pure helium transports the entire amount of nitrogen emitted, and gas chromatography with a thermal conductivity detector (TCD) measures it. The nitrogen analyzer can be calibrated using ultra-high purity acetanilide and EDTA as standards. Using a protein conversion factor, the nitrogen content of the sample is transformed into its protein content.

Procedure: Using automated equipment, samples (about 100–500 mg) are weighed into a tin capsule and added to a combustion reactor. A built-in gas chromatograph measures the amount of nitrogen emitted.

Benefits: No dangerous chemicals are needed. can be finished in three minutes. Up to 150 samples can be automatically analyzed by modern automated equipment.

The need for costly equipment is a drawback. Measures not only protein nitrogen but also total organic nitrogen.

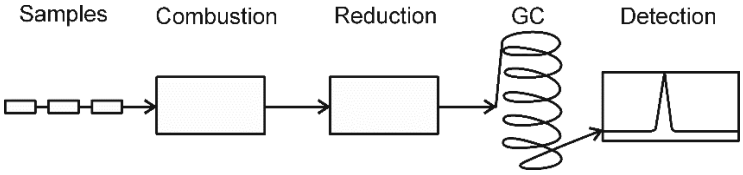

Fig. 1.9 Apparatus for Dumas method.

1.2.5 Digestion, Absorption and Metabolism of Proteins

DIGESTION

In mouth: Mechanical- Mastication Physical- Reacts with Saliva. Proteolytic enzymes acting on proteins are shown in Table 1.2.

Table 1.2 Proteolytic enzymes acting on proteins.

Area	**Mouth**	**Stomach**	**Small intestine**					
Juice	**Saliva**	**Gastric juice**	**Pancreatic juice**			**Succus entericus**		
Enzyme	No proteolytic enzyme	Pepsin	Trypsin	Chymo-trypsin	Carboxy peptidases A & B	Dipeptidases	Tripeptidases	Aminopeptidases
Substrate	Polysaccharides cooked starch	Proteins	Proteoses	Peptones	Dipeptides Tripeptides Polypeptides	Dipeptides	Tripeptides	Large polypeptides
End product	Disaccharides – dextrin and maltose	Proteoses, peptones, large polypeptides	Amino acids			Amino acids		

***Stomach*:** The sole proteolytic enzyme found in gastric juice is pepsin. Gastric juice also contains rennin. However, it is not present in humans.

In the *small intestine*, the proteolytic enzymes of the pancreatic juice and succus entericus break down the majority of the proteins in the duodenum and jejunum.

Pancreatic juice- proteolytic enzymes: Trypsin, chymotrypsin, and carboxypeptidases are all found in pancreatic juice. Because they break the internal bonds of protein molecules, trypsin and chymotrypsin are referred to as endopeptidases.

Enzymes that break down proteins in Succus entericus: The proteolytic enzymes found in the succus entericus are responsible for the last stage of protein digestion. Dipeptidases, tripeptidases, and aminopeptidases are all present.

Final products of protein digestion: Amino acids are the end products of protein digestion and are taken up by the bloodstream from the colon.

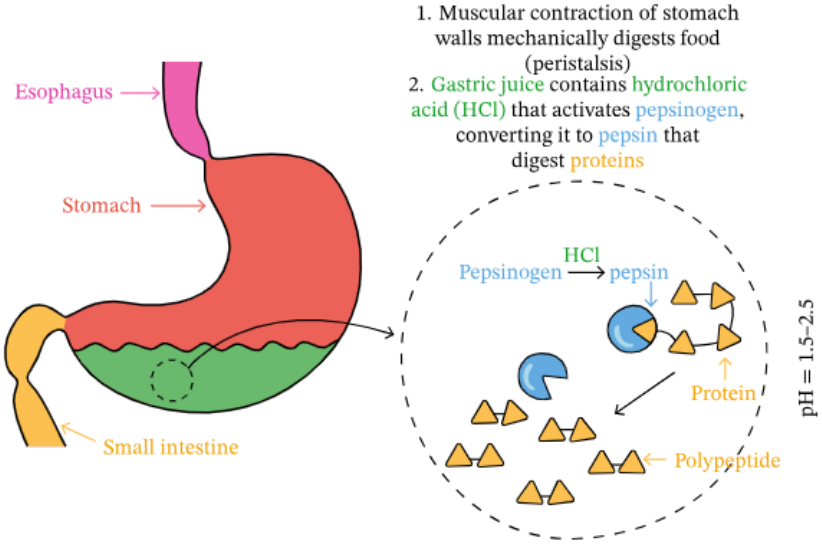

Fig. 1.10 Digestion of protein.

ABSORPTION:

Proteins are absorbed in the form of amino acids from small intestine. The levo amino acids are actively absorbed by means of sodium co-transport, whereas, the dextro amino acids are absorbed by means of facilitated diffusion. Absorption of amino acids is faster in duodenum and jejunum and slower in ileum.

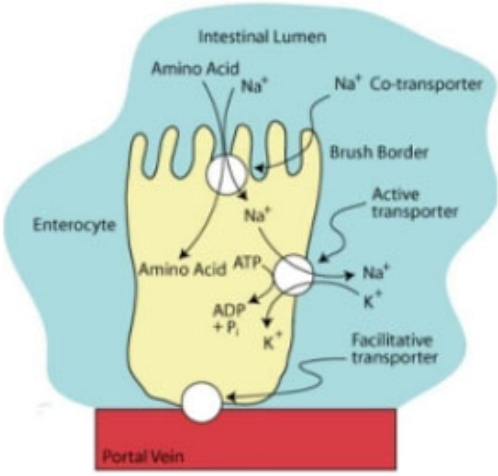

Fig. 1.11 Absorption of proteins.

METABOLISM OF PROTEINS

The excess protein converted to glucose and fatty acids if the energy is less. The excess Amino acids are degraded and excreted from the body by urea cycle. The breakdown of proteins is shown in a schematic picture.

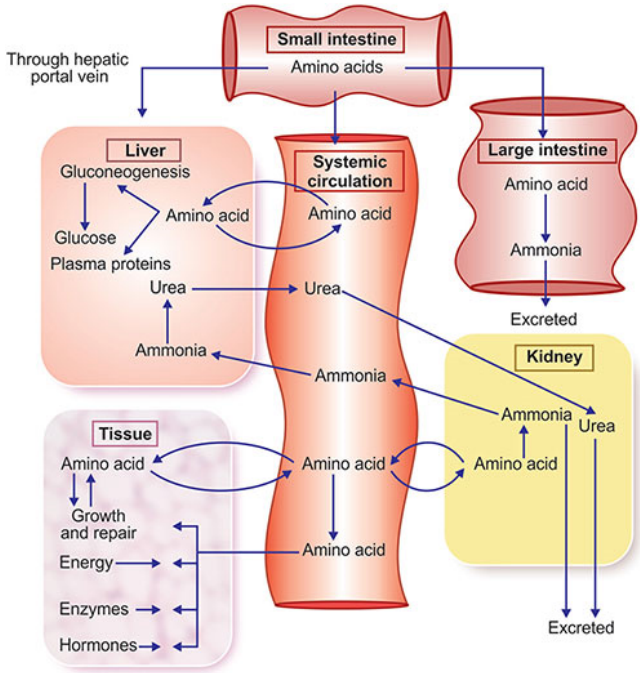

Fig. 1.12 Metabolism of proteins.