UNIT 7 PROTEINS AND ENZYMES

Structure

7.0 OBJECTIVES

After studying this Unit, we shall be able to:

- define proteins and enzymes;
- describe the composition of proteins and enzymes;
- classify proteins and enzymes into different types;
- enumerate the properties of proteins and enzymes; and
- describe the role of proteins and enzymes in food industry.

7.1 INTRODUCTION

In the previous Unit, you learnt about carbohydrates as important constituents of food and their significance in food industry. Another very important constituent of food, proteins are found in all cells and in almost all parts of cell. They contribute to almost half of the body dry weight. Proteins are major organic constituents of protoplasm and a number of extra cellular components. These are important dietary constituents and perform a wide range of functions like providing structure to the body, transporting oxygen and other substances within an organism, regulating the body chemistry etc. Proteins are essential not only as constituents of food but they also have a significant role to play in the processing and preparation of food. This is primarily due to their water binding capacity and ability to coagulate on heating, Proteins find applications as gel formers, emulsifiers and foaming agents etc. This Unit deals with the classification, structure and properties of proteins along with their role in food processing and preparation.

You know that enzymes are also proteins, and they work as catalysts in carrying out the biological reactions. Several enzymes like amylase, invertase, pectinases, proteases etc. find applications in food processing. It is important to learn about the various classes of enzymes and their role in food processing. These aspects are also dealt with in this Unit.

7.2 OCCURRENCE OF PROTEINS

The term protein was coined by Dutch chemist G.T. Mulder in 1839 on the suggestion of Swedish chemist Jons J Benzelius.

The term protein originates from the Greek word *προτεοσ* (proteios), meaning ***of first importance***. These biomolecules are of a large variety and of different sizes and structures. All of these are made up of repeating units or ***blocks*** of amino acids. You will read about amino acids in Sec.7.4. All proteins contain carbon, hydrogen, oxygen, nitrogen and sulphur. Some of them contain small amounts (< 1%) of phosphorus and some a small quantity of iron, copper, or manganese. Generally, a protein has approximately the following composition.

Carbon, 53%; Hydrogen, 7%; Oxygen, 23%; Nitrogen, 16%; and Sulfur, 1%

Proteins constitute most of the nuclear materials of cells and occur in dried seeds of plants more than in plant tissues. The three main sources of proteins include plants, animals and microbes. Let us read where from we get proteins under these categories.

7.2.1 Plant Sources

Gluten: is a protein found in flour, which forms during bread making. The gluten forms network that traps CO_2 created by yeast, giving bread its characteristic texture and air bubbles.

Plant sources of proteins include cereals, lentils/pulses (*dals)*, nuts and oil seeds besides small quantities from vegetables. Cereals like wheat and rice are important sources of protein and are the staple foods of the populations in India. On average, wheat has 12-13% protein while rice has 7-9% protein. Gluten proteins are responsible for the unique bread making property of wheat. Legumes (pulses) and oil seeds are major sources of vegetable proteins. The fresh vegetables do not contain a good amount of protein. The average protein content of the major pulses, oil seeds and some fresh vegetables is given in Table 7.1.

Besides, nuts like cashew nuts, almond nuts, coconuts; walnuts, etc. are the excellent sources of proteins.

Table 7.1: Protein Content of some Pulses, Oilseeds and Fresh Vegetables

		Name	Protein (%)
***Dals* and Pulses**	1	Bengal gram dal	20.8
	2	Black gram dal	24.6
	3	Green gram dal	24.5
	4	Lentil	25.1

		Name	Protein (%)
	5	Dry bean	24.9
	6	Dry pea	19.7
Fresh vegetables	7	Fresh bean	2
	8	Fresh pea	6
	9	Carrot	1
Oilseeds	10	Ground nut	26.7
	11	Soybean	43.2
	12	Sesame	18.3
	13	Cotton seed	19.5
	14	Sunflower seed	12.5

7.2.2 Animal Sources

Proteins are found in animal tissues like muscle, offal and blood. The dietary sources of animal proteins include milk, egg, fish, poultry and meat. The amount of protein in these foods is given in Table 7.2.

The animal proteins have a shorter storage life and therefore plant protein foods are consumed in greater amounts as compared to animal protein foods.

Table 7.2: Protein Content of Some Animal Foods

S.No.	Source	Protein (%)
1	Meat	18-22
2	Milk	3.5
3	Egg white	12
4	Fresh water fish	13-25

7.2.3 Microbial Sources

The proteins are also obtained from microbial sources, i.e. algae, fungi, bacteria, yeast etc. These are termed as single cell proteins (SCP) and are isolated from microorganisms. The isolation of proteins from microorganisms is a cost effective process with good yield.

The term SCP was coined by Prof. Caroll Wilson in 1966.

Fig. 7.1: Various sources of proteins

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1) Arrange the following foods in the increasing order of their protein content.
Spinach, lentils, soybean, pulses, fresh beans, carrots, milk

……………………………………………………………………………

……………………………………………………………………………

7.3 CLASSIFICATION OF PROTEINS

Proteins have wide structural and functional diversity. It is difficult to classify these on the basis of a single property or a characteristic. However, following are some common basis of classifying them.

- Shape and structure
- Products of hydrolysis
- Biological functions

Let us understand each of these in the following subsections.

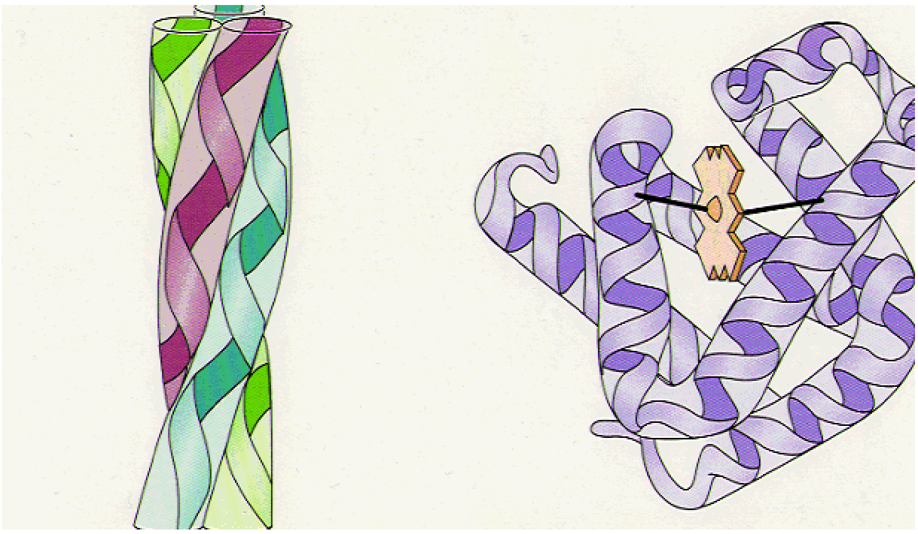
7.3.1 Classification on the Basis of Shape and Size

On the basis of their composition, proteins get different shapes and size which give an indication of their various functions. These can be broadly put into two classes on the basis of their overall shape. These are as follows:

Globular proteins: These contain compactly folded coils of polypeptide chains giving them shape of spheroids or ellipsoids. Examples of this type are albumins, globulins, histones, protamines etc.

Fibrous proteins: This class of proteins looks like fibres or threads. These are insoluble in water and aqueous solutions of acids and bases. These have high mechanical strength. Keratins in hair, actins and myosin in muscles and collagen are examples of this type of protein.

Structures of myoglobin and collagen are shown in Fig.7.2 representing the shape of fibrous and globular proteins respectively.



(a) Collagen **(b) Myoglobin**

Fig. 7.2: Structures depicting fibrous and globular proteins respectively

7.3.2 Classification on the Basis of Products of Hydrolysis

On the basis of the products obtained on hydrolysis, proteins can be classified into three categories viz., simple proteins, conjugated proteins and derived proteins.

Simple proteins: Simple proteins are those which are made of amino acid units, each joined by a peptide bond. Upon hydrolysis they yield only a mixture of amino acids. Following are some of the types of simple proteins.

Table 7.3: Types of Simple Proteins with their Examples

S.No.	Type	Examples
1.	Albumins	Egg albumin, serum albumin, lactalbumin
2.	Gliadin	Tissue globulin, serum globulin
3.	Gliadins	Wheat gliadin, hordein (barley) etc.
4.	Albuminoids	Keratin of hairs, skin, egg shell and bones, elastin, collagen of tendons, ligaments and bones.
5.	Histones	Globin of haemoglobin
6.	Protamine	Salmine from the spermatozoa of salmon fish

Conjugated Proteins: Conjugated proteins are composed of simple proteins combined with non protein substances. The non protein substance is called **prosthetic group** or **cofactor**. Following are some of the types of conjugated proteins.

Table 7.4 Types of Conjugated Proteins and their Examples

S.No.	Types	Example
1.	Chromoproteins	Haemoglobin, in which the prosthetic group is iron
2.	Phosphoproteins	Casein in milk and vitellin in egg yolk containing phosphoric acid as prosthetic group
3.	Lipoproteins	HDL (high density lipoprotein), LDL (low density lipoprotein) and VLDL (very low density lipoproteins), have lipids as the prosthetic groups
4.	Glycoprotein	Ovomucoid of egg white containing a carbohydrate moiety
5.	Nucleoproteins	Ribosomes and viruses contain nucleic acids
6.	Metalloproteins	Alcohol dehydrogenase- a Zn containing enzyme
7.	Mucoproteins	Follicle stimulating hormone, β-ovomucoid

Derived Proteins: These are not naturally occurring proteins and are obtained from simple proteins or conjugated proteins by the action of enzymes and chemical agents, heat, mechanical shaking, UV or X-rays.

It includes the following types:

1. **Primary** e.g., myosin, fibrin
2. **Secondary** e.g., peptones, peptides, proteoses etc.

7.3.3 Classification on the Basis of Biological Functions

The involvement of proteins in different functions also makes it a basis for their classification. The different classes along with the functions performed and examples are summaries in Table 7.5.

Table 7.5: Types of Proteins on the Basis of Functions Performed

S.No.	Type	Function	Example
1	Enzymes	Catalytic activity	Kinases, dehydrogenases
2	Storage proteins	Store amino acids	Myoglobin, ferritin
3	Regulatory proteins	Coordinate body activities	Insulin, glucagons

S.No.	Type	Function	Example
4	Structural proteins	Give support and structure	Keratin, collagen
5	Defensive/Protective proteins	Protect against diseases	Immunoglobulins, antibodies
6	Transport proteins	Facilitate import of nutrients into cells or releases of toxic products into surrounding medium	Haemoglobin
7	Contractile and Mobile proteins	Participate in contractile processes e.g. movement of muscles	Actin, myosin

Emulsoids and Suspensoids

In yet another way, the proteins are classified on the basis of their precipitation as emulsoids and suspensoids. **Emulsoids** are those proteins which stay in solution with lot of bound water and are not coagulated by mild acid. On the other hand, the **suspensoids** are those proteins that stay suspended due to the repulsion between charges. These are coagulated at their isoelectric point or by the charge neutralization. Milk is a good example as it contains both of these types. On mild acidification (pH = 4.7-5.3), the casein (suspensoid) separates out and the other proteins remain in the solution and can be separated by filtration. These are called milk serum or **whey** proteins and contain a number of proteins like lactoalbumin and lactoglobulin etc. These may be further separated by salting out with ammonium sulphate or magnesium sulphate.

Check your understanding of the classification of proteins by answering the following questions.

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1) Fill in the blanks spaces with appropriate words.

i) The compact and folded structures of proteins are put into the category of ………………. proteins whereas …………………proteins have a thread like structure.

ii) Proteins which give support to the tissues are called …………proteins.

iii) Haemoglobin is an example of a …………………………. protein.

iv) Proteins obtained as a result of hydrolysis of proteins by the action of chemical agents, heat etc. are called ………………………..proteins.

7.4 STRUCTURE OF PROTEINS

It was mentioned in Sec.7.2 that structurally proteins are polymers of α- amino acids which join together through peptide bonds. These polymeric molecules acquire different arrangements depending on their composition and the nature of amino acids constituting them. These arrangements are stabilized with the help of different types of interactions. The protein structure can be understood in terms of four hierarchical levels. Let us learn about amino acids, their combination to give peptides and proteins and the structure of the proteins so obtained.

7.4.1 Amino Acids

Amino acids are organic molecules and as the name suggests, an amino acid consists of an **amino group** and a **carboxyl group**. The carbon atom which has these two groups attached to it has a hydrogen atom, and a distinctive R group (called side chain) bonded to it. This carbon is called the **α-carbon** because it is adjacent to the carboxyl group. The general formula of an α-amino acid can be represented as shown in Fig. 7.3.

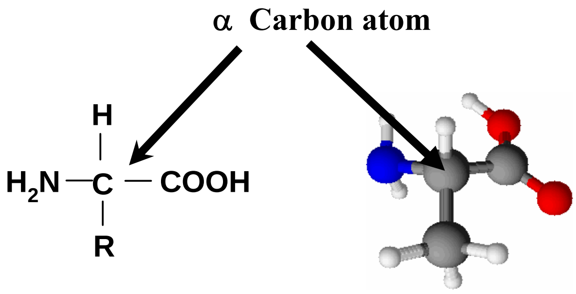


Fig. 7.3: General structure of an α-amino acid

More than twenty natural amino acids are known. Different amino acids contain different side chains i.e.,-R groups, varying in size, shape, charge, hydrogen-bonding capacity and chemical reactivity.

Essential and Non-essential Amino Acids

On the basis of their source in the living system, the amino acids are classified into essential and non-essential amino acids. Of the twenty standard amino acids, in case of humans, more than half of these can be made by the body itself, while the others must come from the diet. The former are called the **non-essential amino acids** and the latter are called the **essential amino acids**. The essential amino acids (for human beings) are isoleucine, leucine, valine, lysine, methionine, phenylalanine, threonine and tryptophan. The classification of an amino acid as essential or non-essential does not reflect its importance as all the twenty amino acids are necessary for normal functioning of the body. It simply reflects whether or not the body is capable of synthesizing a particular amino acid. The requirement of essential amino acids per kilogram of the dietary protein is called the **reference pattern** of the amino acids and acts as a standard to determine the quality of the protein being consumed.

The requirement of essential amino acids
(g per kg dietary protein)

Isoleucine :	42
Leucine :	48
Lysine :	42
Methionine :	22
Phenylalanine:	28
Threonine:	28
Tryptophan:	14
Valine:	42

7.4.2 Peptides

Two or more, similar or different amino acids combine to give a condensation product with elimination of a water molecule. The product is called the **peptide.** The amide linkage joining the carboxyl group of one amino acid to the amino group of another amino acid is called a **peptide bond.** Two amino acids join to form a di-peptide, three form a tri-peptide, four form a tetra-peptide and so on. Accordingly, these contain one, two, three and more number of peptide bonds. The reaction showing the formation of a peptide is given below.

In a peptide, the amino acid whose carboxyl group participates in the formation of the peptide bond has a free amino group, and is called as the **N- terminal** of the peptide. Correspondingly, the amino acid with a free carboxyl group is called the **C-terminal** of the peptide. The N- terminal is conventionally written to the left while the C-terminal is written to the right.

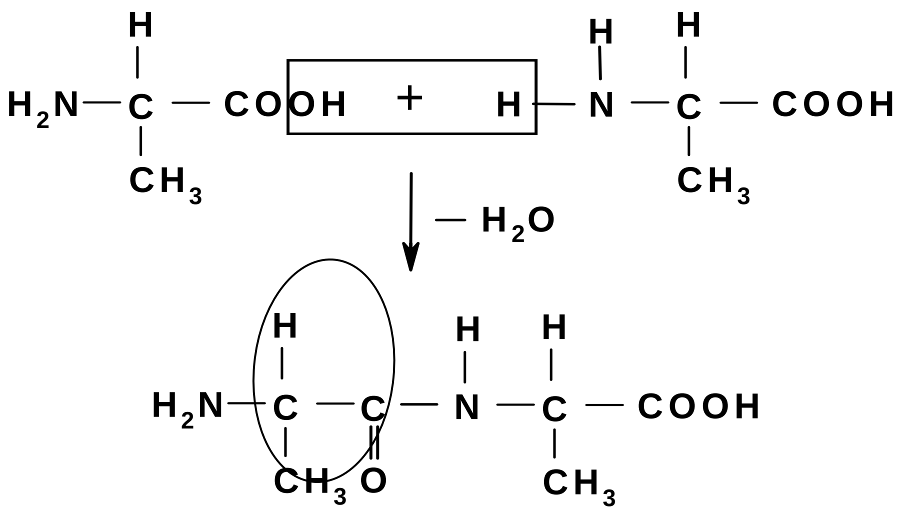


Fig. 7.4: Formation of a Peptide Bond

The number of amino acids in a peptide may vary from 2 to hundreds. Peptides containing 3-10 residues are called **oligopeptides**. When the number is very large then it is called a **polypeptide**. A polypeptide with more than say about 50 amino acids is called a protein. You shall read in the next subsection about the structure of proteins.

7.4.3 Structural Organization of Proteins

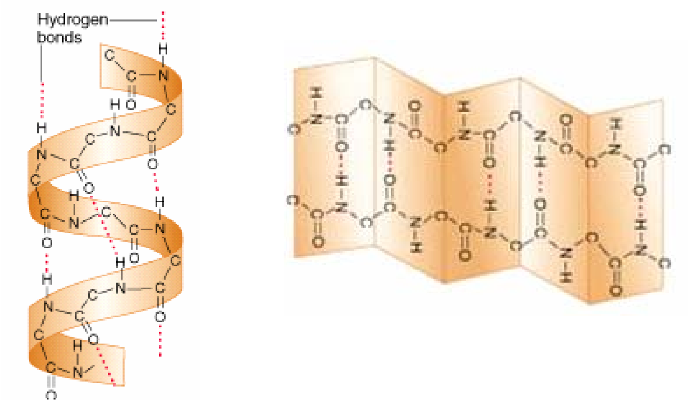
The wide variety of functions performed by proteins is due to the possibility of formation of a large number of proteins from a given number of amino acids. The large polymeric molecules of proteins acquire intricate three-dimensional structures. The structural organization of proteins can be seen at four levels. These are, primary (1°), secondary (2°), tertiary (3°) and quaternary (4°) structures. These are explained below:

The complete covalent structure of the polypeptide constitutes its primary structure.

Primary structure: The primary structure of a protein is the sequence of amino acid residues in a completely assembled polypeptide chain. The chain begins with the first amino acid residue at the N-terminal end and progresses in sequence to the last amino acid at the C-terminal end. The primary structure of a protein contains all the necessary information required for the manifestation of higher levels of a three dimensional structure.

Secondary structure: The secondary structure of a protein is related to the way in which the chain of amino acids either twists, folds back upon itself to form a helical, sheet like or a random arrangement. The first two are respectively called the **α-helix** and the **β- pleated sheet** structures. The α-helix and the β- pleated sheet structures are characteristic of fibrous proteins like keratin, silk fibroin or collagen. The secondary structures of proteins are shown in Fig.7.4.

Tertiary Structure: The tertiary structure of the protein refers to the overall three dimensional structure of the protein. The secondary structures of different regions of the polypeptide chain put together give an overall shape to the polypeptide. This is called the **native structure** of the protein. In this tightly folded structure, the side chains of the amino acid residues with polar groups are exposed to the surface and non-polar groups are buried inside. The stability of the tertiary structure is maintained by hydrogen bonds, attraction between positively and negatively charge side groups, van der Waal forces, and polar and non-polar associations.



(a) **(b)**

Fig. 7.5: Secondary structures of proteins (a) α-helix, (b) β- pleated sheet

Quaternary Structure of Proteins: Many proteins exist naturally as aggregates of two or more polypeptide chains. Each of these polypeptides is usually folded into an apparently independent conformation. These aggregated structures are called as quaternary structures of the proteins. Structure of haemoglobin depicting the quaternary structure of proteins is given in Fig. 7.6.

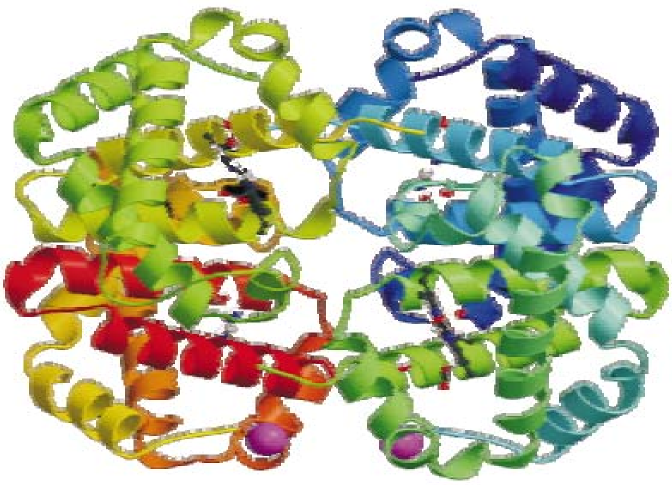


Fig. 7.6: Structure of haemoglobin showing the quarternary structure of proteins

Check your understanding about the structure of proteins by answering the following exercise.

Check Your Progress Exercise 3

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1) Essential amino acids are called so because

i) These are essential for the growth of the body.

…………………………………………………………………………
…………………………………………………………………………
…………………………………………………………………………

ii) The body cannot synthesize them and have to be taken in diet.

…………………………………………………………………………
…………………………………………………………………………
…………………………………………………………………………

iii) These are essential for the synthesis of proteins.

…………………………………………………………………………
…………………………………………………………………………
…………………………………………………………………………

iv) These are included in the list of 20 amino acids.

…………………………………………………………………………
…………………………………………………………………………
…………………………………………………………………………

2) Match the contents of column A and column B correctly.

Column A	Column B
i) Sequence of amino acids in protein protein	i) Quaternary structure of
ii) Polypeptide with three peptide bonds	ii) Primary structure of protein
iii) Combination of two or more polypeptides in protein	iii) Tetrapeptide

Electrophoresis: The migration of charged colloidal particles or molecules through a solution under the influence of an applied electric field usually provided by immersed electrodes.

7.5 PROPERTIES OF PROTEINS

The properties of the proteins depend on the composition and the structure of the protein. You have already learnt that the side chains of the amino acids constituting the protein vary a great deal in terms of their nature and functional groups. The properties of proteins are therefore related to how these groups are arranged in proteins. The main physico-chemical properties are discussed below:

7.5.1 Physico-chemical Properties

Isoelectric point (pI)

As you are aware, the amino acids and therefore the proteins can act both as acids or bases, they are said to be **amphoteric** in nature. Due to the presence of charged groups they have an ability to migrate in presence of an electrical field. The nature and number of charges depend upon the pH of the medium and at a specific pH, the protein exists in an electrically neutral (zwitterions) form. This pH is known as **isoelectric point** or **pI** of the protein. Since at this pH the protein has an equal number of positive and negative charges, the net charge of the protein is zero. Therefore, the protein does not move to either electrode when placed in an electric field. At a pH lower than the **pI** the protein would move towards cathode while at a pH greater than **pI** it would move towards the anode. Since the charges on different proteins at a given pH are different, these can be separated in a process called **electrophoresis**.

Solubility

Solubility of proteins is influenced by the nature of solvent, pH, temperature, etc. Globular proteins are generally more soluble in aqueous medium in comparison to elongated fibrous proteins such as keratins. Insolubility of proteins is directly related to its denaturation i.e. aggregation. Aggregation, in turn, influences the effectiveness in food processes like gelation, emulsification and foaming.

Precipitation

Proteins may be precipitated without getting denatured at isoelectric pH, in presence of salts of heavy metals or in presence of a nonpolar solvent like chloroform. In the process, the electrostatic attraction between the ions of proteins gets enhanced and thus facilitating their aggregation and precipitation.

Let us now see how the physicochemical properties of proteins influence their functional properties and contribute to the desirable properties of food.

7.5.2 Functional Properties of Proteins

You are aware that the functional properties of the food constituents refer to their attributes that make them useful for the food besides being nutritionally important. These are the physico-chemical properties which influence the characteristics of foods, e.g., texture, taste, appearance, etc. Emulsification, viscosity, formation of foams and gels, binding to fats or water molecules etc. are some of the functional properties of proteins. An understanding of these properties helps the food technologist to improve the appearance, taste and texture of the food by exploiting them.

These properties are related to the surface properties of the proteins or are the result of the interaction of proteins with its own molecules or water molecules. Accordingly, the functional properties of proteins are classified into the following three categories.

- Hydration properties : related to protein-water interaction.
- Structure formation properties : related to protein-protein interaction.
- Surface properties : related to protein-interface interaction.

Hydration Properties (protein-water interactions)

We know that most of the foods are hydrated to some extent and the behaviour of the proteins in it are influenced by the presence of water and water activity. A number of the properties of proteins like swelling, solubility, precipitation, viscosity, gel formation etc. are related to the interaction of protein molecules with water molecules. These are collectively referred to as the **hydration**

properties. The hydration takes place in a sequence of steps and is said to be progressive in nature. Different hydration properties like water absorption, swelling, wettability, water retention, viscosity and solubility are dependent on the extent of hydration. Highly soluble proteins find applications where emulsification and whipping etc. are important. Soluble protein powders with high initial water absorption capacities give relatively high viscosities and find application in fluid foods like beverages, soups, sauces and creams.

The denatured protein molecules can aggregate to form an ordered network called a **gel**. This is a result of a balance between protein-water and protein-protein interactions. These gels can hold other food ingredients and find applications in preparation of yogurt, tofu and bread dough etc. The gelation of proteins may be exploited for water and fat absorption, thickening, and for stabilizing of emulsions and foams.

Proteins provide the basis for the texture and structure of many food products as a fibrous constituent per say or in the soluble form which form a chewable fibrous product by a series of texturization processes.

Structure Formation Properties (protein-protein interactions)

As mentioned above, the formation of a gel involves protein-protein interactions besides the role of water. It involves interplay of attractive and repulsive interactions between different polypeptide chains. The gelation plays a role in water absorption, water control and thickening etc. Another crucial property of the proteins is **dough** formation. You know that the dough is nothing but a strongly cohesive and viscoelastic mass formed from gluten, the proteins present in wheat grain endosperm. This also has contributions from the starch granules, some lipid and other soluble proteins. The dough is stabilized by hydrogen bonding between different amino acid side chains, hydrophobic interactions between apolar amino acids and numerous disulphide (-S-S-) cross linkages. In other words we can say that dough formation is a consequence of **protein-protein interactions**.

Viscoelastic: having flow-resistant as well as stretchy properties

Surface Properties (protein-interface interactions)

Proteins are surface active agents. You are aware that in the three dimensional structure of the proteins in aqueous solutions, the hydrophobic amino acids are buried inside in the bulk, while the amino acids with hydrophilic side chains are on the surface. The active surface of the proteins can interact with a wide variety of molecules like water, lipids, volatile flavours, carbohydrates and even other protein molecules. Due to this, the proteins find extensive applications as emulsifiers in deserts, whipped creams and spreads etc. The proteins also stabilize emulsions and contribute to the physical and rheological properties like viscosity, thickness and elasticity etc. Proteins are good at producing foams in cakes ice creams etc. where these exist with varied textures.

Rheology: study to deformation and flow of matter

The surface properties of the proteins may be exploited in binding the desirable flavours on to it, though sometimes it may be disadvantageous as these can also bind undesirable flavours causing to initiate de-odourisation. In addition, the active surface of the proteins may provide site for the binding of a number of other substances like pigments, synthetic dyes and substances with mutagenic activity. These binding interactions may lead to adverse nutritional or toxic consequences.

7.5.3 Food Applications of Protein Concentrates, Isolates and Hydrolysates

The protein concentrates and isolates are the products obtained by selectively removing the non-protein ingredients from a protein source so as to obtain a product that is rich in its protein content. The procedure is such that the nutritional and functional properties of the proteins are retained. These find extensive applications in different formulations so as to impart nutritional as well as functional properties to them. The protein hydrolysates, on the other hand are the products obtained on treating the proteins with enzymes that hydrolyze them. These products consist of mixtures of amino acids and small products. These hydrolysates have superior nutritional qualities and higher bioavailability.

The **Soy Protein Concentrates** (**SPC**) are prepared from high quality, sound, clean, dehulled soybean seeds by removing most of the oil and water soluble non-protein constituents. It contains not less than 70% protein on moisture free basis. The most important application of SPC is in food industry primarily in comminuted meat, poultry and fish products like patties, sausages and fish sticks etc. The SPC increase the water and fat retention in these products. Besides these, the SPC are also used as stabilized dispersions in milk, beverages and simulated dairy products like sour cream analogues.

The **Soy protein isolates (ISP)** are the major proteinaceous fraction obtained from dehulled soybeans and contain at least 90% protein on moisture free basis. The major food applications of ISP's are in meat products as a meat replacer, in sea foods like fish sausages and in cereal, bakery and dairy products.

The **Whey Protein Concentrate** (WPC) is a highly nutritious product obtained by selectively removing water, minerals and lactose from whey. These are removed by physical separation techniques like precipitation, filtration or dialysis. The WPCs find extensive applications in baked goods and contributes towards the product browning, crust and flavour development. The concentrates with high viscosity are used in ice creams, processed cheese and fresh dairy applications.

The **protein hydrolysates** are obtained by hydrolysis of food proteins by using proteases like chymotrypsin, papain, thermolysin and trypsin. The hydolysis process alters the composition and functional properties of the proteins like gelation, foaming and emulsification. The hydrolysates are used in soups and sauces and are important for the people who cannot digest solid foods.

After having an understanding of the structure and properties of proteins it will be easy for you to understand the behaviour of enzymes and their role in food industry. Let us read about enzymes in the section that will follow exercise.

Check Your Progress Exercise 4

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

Complete the statements given below with appropriate answers.

i) The net charge of proteins at this pH is zero……………………….
ii) It is due to the presence of amino acids with hydrophilic

side chains that proteins possess this food application ………………….

iii) Food processes like gelation, emulsification and foaming relate to this property of proteins ………………………………

7.6 ENZYMES

Enzymes are the proteins that catalyze biochemical reactions. Study of the important biochemical reactions was started many years ago, from the time of Louis Pasteur, who for the first time demonstrated the fermentation of glucose by yeast. The catalytic agent of yeast cell was subsequently identified and named as **ferment**. An enzyme can be a large protein made up of several hundred amino acids, or several polypeptides that act together as a unit. Enzymes have molecular weights ranging from 10,000 to 2,000,000.

Enzymes may sometimes have a non-protein component attached to the protein part. This may be an organic compound or a metal ion. While the former is called as **coenzyme**, the later is best known as **cofactor**. The protein part and non-protein part together form the **holoenzyme**, as illustrated in Fig. 7.6. Sometimes, the non-protein part remains so tightly bound to the protein part that it cannot be dissociated. Such non-protein part is called as a **prosthetic group**. When the non-protein component dissociates from the protein part, the enzyme loses its catalytic function and is called an apoenzyme as can be seen in the Fig. 7.7.

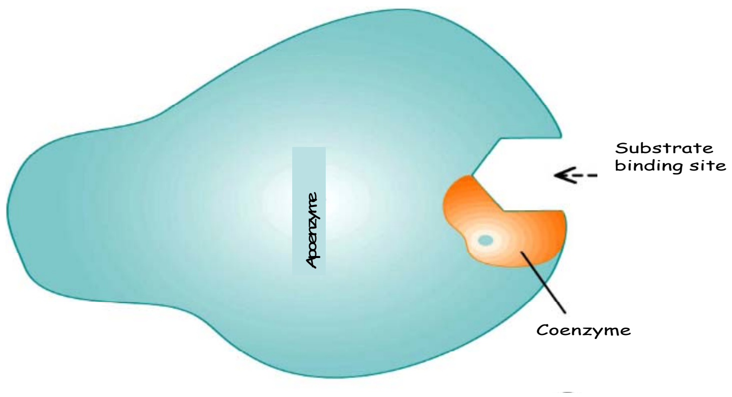


Fig.7.7: Schematic representation of enzyme with protein and non-protein part

7.5.1 Nomenclature and Classification of Enzymes

Conventionally, the trivial or common names of the enzymes were derived either from the names of the substrate they acted on, or on the reaction they catalyzed or both. These ended in a suffix '**–ase**' for example, *urease* is an enzyme acting on urea. However as the number of enzymes known went up, a need to have a formal scheme of nomenclature was felt and accordingly, there is an enzyme nomenclature system in place. According to this system, the enzymes are grouped into six classes; each class is subdivided into several subclasses, which are further subdivided. Every enzyme is characterized by a code number.

The code numbers of the enzymes are prefixed by **EC (Enzyme Commission)** and contain four numbers separated by points, with the following meaning:

(a) the first number shows to which of the six main classes an enzyme belongs
(b) the second figure indicates the subclass
(c) the third figure gives the sub-subclass, and
(d) the fourth figure is the serial number of the enzyme in its subclass.

For example, the EC number of the enzyme catalase is EC 1.11.1.6

The major classes and the type of reaction catalyzed by them are given below:

Oxidoreductases: The enzymes of this class (EC 1) catalyze oxidation-reduction reactions. Common names include *dehydrogenases, oxidases, reductases* and *catalases*.

Transferases: These are the enzymes (EC 2) which catalyze the transfer of a group, e.g., a methyl or glycosyl group, from one compound to another. In many cases, the donor is a cofactor (coenzyme) carrying the group to be transferred. Common enzymes of this group include *acetyltransferase, methylase, protein kinase* and *polymerase*.

Hydrolyases: These enzymes (EC 3) catalyze the hydrolytic cleavage of C−O, C−N, C−C and some other bonds, including phosphoric anhydride bonds. Their trivial names are formed by adding the suffix 'ase' to the substrate which they hydrolyze. Examples include *protease, nuclease*, *phosphatase*.

Lyases: These enzymes (EC 4) cleave C−C, C−O, C−N and other bonds by elimination, forming double bonds or conversely adding groups to double bonds. Common examples include *decarboxylase, aldolase, dehydratase* (if water is eliminated) or hydro-lyase (if the reverse reaction is more important or the only one which can be demonstrated.

Isomerases: These enzymes (EC 5) catalyze geometric or structural changes within a molecule. According to the type of isomerism, they may be called *racemases, epimerases, cis-trans isomerases (5.2), isomerases, tautomerases, mutases or csycloisomerases.*

Ligases*:* These are also called synthetases (EC 6) and catalyze the linkage of two molecules coupled with the hydrolytic breakdown of a pyrophosphate bond in ATP or an analogous compound. The bonds formed are often high energy bonds. The second figure in the code number indicates the bond formed, e.g. C−O (6.1), C−S (6.2) etc. Examples include *peptide synthase, aminoacyl-tRNA synthetase, DNA ligase* and *RNA ligase*.

Monomeric and Oligomeric Enzymes

On the basis of polypeptide units constituting the enzyme, these are categorized into **monomeric** and **oligomeric** enzymes. As is evident from the name itself, the monomeric enzymes contain a single polypeptide unit, while the oligomeric enzymes contain two or more such units. The monomeric enzymes are primarily *proteases* i.e., they catalyze the hydrolysis of peptide bonds of other protein molecules. The examples being *serine protease, thiol protease* etc.. The oligomeric enzymes on the other hand, include a vast majority of enzymes. The examples being, *lactose synthase*, *lactate dehydrogenase* and *pyruvate dehydrogenase etc.*

Check Your Progress Exercise 5

Note: a) Compare your answers with those given at the end of the unit.

1) Fill in the blank spaces in the following.

   a) The tightly bound non-protein part of enzymes is called the …………………...
   b) The enzymes responsible for cleavage of bonds and formation of double bond belong to the class ………………………………
   c) ……………………………is an example of an enzyme catalyzing hydrolytic cleavage in biochemical reactions.
   d) ………………………… are also called …………………… due to their involvement in linking two molecules and forming bonds.

7.7 ENZYME UTILIZATION IN FOOD INDUSTRY

The biocatalytic activity of the enzymes is of immense importance in food technology. Many reactions catalyzed by enzymes contribute towards the desired attributes of the processed food. At the same time, a number of enzymatic reactions produce undesirable results. Further, the enzymes may be used in industry as components of living cells or after isolation in free or immobilized forms. Let us take up some of the applications of enzymes in the food industry.

Bread making: In baking bread, the preliminary process involves the mixing of wheat flour (mainly starch and proteins) with yeast and water. Starch gives glucose, maltose and some oligosaccharides. Glucose and maltose are then metabolised by the enzymes in yeast, and carbon dioxide is formed. This CO_2 then disintends the protein framework of the dough, ready for baking.

Blanching: A hot-water or steam treatment of raw foodstuffs to inactivate enzymes which otherwise might cause quality deterioration, particularly of flavour, during processing or storage.

Vegetable preservation: You know that the fresh vegetables contain a number of enzymes. In the process of preservation of these vegetables, the enzymes must be inactivated; else the vegetables would soon get deteriorated. This inactivation is achieved by a process called **blanching,** blanching helps in inactivating the enzymes in vegetables. Therefore it is essential blanc the vegetables before their preservation.

Ale: A fermented alcoholic beverage containing malt, similar to beer, but heavier.

Stout: A strong, very dark beer.

Brewing industry: Brewing refers to the manufacture of beer and other malt beverages like ale and stout. The process involves germination of barley under moist conditions which is then dried and the malt so obtained is the main starting material. The reserve starch is broken down by the amylase present to give, among other products, glucose, maltose and soluble starch. The grains are then roasted and the soluble component called **wort** is extracted with water. This wort is then fermented in presence of yeast to obtain alcohol. The alcohol thus accrued is separated by distillation for making different types of alcoholic drinks.

Whey: The watery part of milk, separated from the curds, as in the process of making cheese.

Cheese production: involves the conversion of the milk protein, k-casein to paracasein by a defined, limited hydrolysis, catalyzed by chymosin (rennin). Rennin the gastric enzyme of the calf is used in the form of a crude extract, powder or paste. In the presence of Ca^{2+}, paracasein clots and may be separated from the whey after which the clot is allowed to mature under controlled conditions to form cheese.

Tenderizing of meat: when meat is cooked fresh it is quite tender. However, with the passage of time the muscle becomes inextensible and is tough when cooked. The meat can be tenderized with the help of **proteolytic** enzymes e.g. proteases. For this purpose, the meat is kept at low temperature (~ 4°C) for many weeks whereby the enzymes present in the meat tenderize it. This process can be hastened by dusting proteolytic enzymes over the meat before frying or boiling it.

Proteolytic enzymes: catalyzing the hydrolysis of peptide bonds

Enzymes find a number of application in food industry by way of immobilized enzymes about which you will study in the next subsection after answering the following SAQ.

Check Your Progress Exercise 6

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1) Choose the correct answer for the following.

Proteolytic enzymes are able to tenderize meat because

i) these enzymes work at low temperatures.
ii) these enzymes do not take very long for catalyzing the reaction.
iii) the enzymes can catalyze the hydrolysis of peptide bonds.
iv) the meat gets softened after keeping it as such for sometime.

7.7.1 Immobilized Enzymes

The enzymes used to catalyze a given reaction in food processing normally get inactivated in the process and cannot be reused. It was attempted to separate them from the product and put to use again. It could be achieved by attaching the enzyme to a water-insoluble solid support, called the **matrix.** This is called **enzyme immobilzation.**

Immobilised enzymes have been defined as enzymes that are physically confined or localised, with retention of their catalytic activity, and which can be used repeatedly and continuously

You must have observed that if you keep a cut apple exposed to air for some time it starts turning brown. A wrong notion associated with this type of browning is that the change is due to the presence of iron in that particular fruit or vegetable. This is actually incorrect and has different reasons. In recent past the immobilized enzymes have become an indispensable tool in food processing. The immobilization of the enzyme can be achieved by chemical or physical methods. In chemical methods, the enzymes are bound to a solid support through covalent bonding. In case of physical methods, the enzymes are either adsorbed on the solid support, entrapped in gels or are encapsulated behind semi-permeable membranes. The enzymes cannot be recovered from the immobilized enzymes, prepared by chemical means whereas the physical immobilization of the enzymes is reversible.

There are several of advantages of immobilized enzymes. Some of these are given below:

- Easy recovery of the enzyme after the process: The immobilized enzymes are added to the reaction mixture and removed by filtration after the reaction is over. This leads to cost efficiency.
- The greater efficiency and control of their catalytic activity,
- Reusability of the enzyme,

- Improved thermal stability of the enzyme allowing higher processing temperatures.

Treatment of corn syrup with glucose *isomerase* gives high fructose syrup that is 50 per cent more sweet. This is a result of conversion of glucose to fructose by the enzyme. The high fructose syrup finds application in the manufacture of aerated drinks. Nowadays the immobilized *glucose isomerase* is used on a commercial scale for the conversion of glucose to fructose.

7.7.2 Enzymatic Browning

You must have observed that when you keep the cut apple exposed to air for sometimes, it starts turning brown. A wrong notion associated with this type of browning is that it is due to the presence of iron in that particular fruit of vegetable. This is actually incorrect. It has different reasons. You will read and understand it in the following paragraphs.

The browning of some cut fruits and vegetables is called **enzymatic browning** and is due to the activity of a group of enzymes called *phenolases*. The enzymatic browning is a result of two reactions. One being the conversion *o*-dihydroxyphenols to *o*-quinones and the other is hydroxylation of some monohydroxyphenols to dihydroxyphenols. The browning of certain fruits on cutting is due to the polymerization of *Roth* quinines which are formed in the enzymatic reaction.

All the fruits do not brown on cutting. This may be due to the absence of the browning enzymes or that of the suitable substrates. You would agree that the browning is not a desirable phenomenon therefore we need some kind of intervention to retard or prevent it. Applying lemon juice on cut banana or dipping the cut fruits in ascorbic acid or in dilute salt solution are some such interventions. These help by blocking the contact with air or by reducing the enzymatic activity.

Check Your Progress Exercise 7

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1) Tick mark (√) in front of the right statement(s) and mark (X) in front of wrong statement(s) given below.
   i) Enzyme catalysis is an expensive technique because of inability to recover the enzyme used in a process.
   ii) Immobilisation of enzyme is one technique to make the use of enzymes cost effective.
   iii) The recovery of enzymes after enzyme immobilisation is very difficult
   iv) All the types of fruits and vegetables show enzymatic browning

7.8 LET US SUM UP

Proteins are polymeric bimolecular present as important components of plant and animal cells. The main plant sources of proteins include cereals, pulses and oilseeds. The animal sources include meat, poultry and fishes. Proteins are also obtained from microbial sources. The proteins obtained from microbial sources are called single cell proteins.

Proteins are classified on the basis of their shape and size, product of hydrolysis, and biological functions. These are made up of amino acids that consist of an amino and a carboxylic group. The properties of proteins, therefore, reflect the reactions related to both the groups. The amino acids combine together through peptide bonds to form peptides. The peptides may be di, tri, tetra, and so on depending upon the number of amino acids combining. When the number of amino acids is very large, polypeptides are formed. Polypeptides with more than 50 amino acids form protein.

The structural organization of proteins has four levels via, primary, secondary, tertiary and quaternary. The primary structure is the chain of amino acids while other structures are a result of folding of the chain to give rise to various shapes and sizes of proteins.

Proteins find a number of food applications. These are used in emulsification gelation, foaming etc during food processing and preparation. Proteins have an important role in the form of enzymes acting as catalysts in the biochemical reactions. Enzymes initially were named after the substrate or the reaction catalyzed by these. Later these were systematically named and classified according to the Enzyme Commission rules. Enzymes find a number of food applications e.g. bread making, vegetable preservation, brewing etc. They find application in the form of immobilized enzymes also.

7.9 KEY WORDS

Albumins : A class of simple, water-soluble proteins that can be coagulated by heat and are found in egg white, blood serum, milk, and many other animal and plant tissues.

Blanching : A partial precooking by plunging the food into hot water (82-95°C) for ½-5 min. Fruits and vegetables are blanched before canning, drying, or freezing, to soften the texture, remove air, denature enzymes that may cause spoilage when frozen, and remove undesirable flavours.

Enzymatic Browning : Enzymatic browning is a chemical process involving polyphone oxidize or other enzymes that create melanin's, resulting in a brown color. Enzymatic browning is an important color reaction in fruit, vegetables, and seafood. Enzymatic browning of fruits and vegetables creates heavy economic losses for growers.

Essential Amino Acids : An amino acid that must be obtained from the diet so that the body can synthesize vital proteins. The nine essential amino acids are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and histidine.

Immobilized Enzyme : An enzyme which is attached to an inert, insoluble material such as sodium alginate. This can provide increased resistance to changes in conditions such as pH or temperature. The techniques for immobilization include simple absorption, ionic to covalent binding.

Isoelectric Point : The pH value of the dispersion medium of a colloidal suspension at which the colloidal particles do not move in an electric field.

Tenderization : To make (meat) tender, as by marinating, pounding, or applying a tenderizer. Natural tenderizing is caused by the action of enzymes already in tissues. This effect can be enhanced by quick freezing before rigor mortis sets in, and by hanging the meat at the proper temperature for the proper time, especially just before cooking. also called conditioning.

7.10 TERMINAL QUESTIONS

1) Which amino acid will be at the C terminal and which well be at N terminal in tripeptide valylisoleucinylalanine?
2) What is meant by single cell proteins? What are their advantages?
3) Define and distinguish simple, conjugated and derived proteins.
4) What are the four organizational structures of proteins. What is the difference amongst all of these?
5) What do you understand by the functional properties of proteins? How are these depicted in the foods?
6) Define the terms enzymes, coenzymes, cofactors and prosthetic groups.
7) Define immobilized enzymes and give an example of its application in food industry.

7.11 ANSWERS TO CHECK YOUR PROGRESS

Check Your Progress Exercise 1

1) spinach > carrot > fresh beans > milk > lentils > soybean

Check Your Progress Exercise 2

1) i) globular, fibrous
   ii) structural
   iii) transport
   iv) derived

Check Your Progress Exercise 3

1) ii)
2) i) – ii), ii) – iii),
   iii) – i)

Check Your Progress Exercise 4

1) i) Isoelectric point
   ii) Emulsifying property
   iii) Solubility and Precipitation

Check Your Progress Exercise 5

1) a) prosthetic group

b) lyases
c) Protease / nuclease / phosphatase
d) Ligases, synthetases

Check Your Progress Exercise 6

1) iii)

Check Your Progress Exercise 7

1) i) √ ii) √ iii) x iv) x

7.12 ANSWERS TO TERMINAL QUESTIONS

1) Valine will be at the N terminal and alanine at C terminal.

2) Single cell proteins are those obtained from microbial sources. These have advantages of good yield and cost effectiveness.

3) Simple proteins are formed only by amino acids, conjugated have some non-protein part and the derived are the one obtained as hydrolytic products of any of the former two.

4) The four organizational structures are primary, secondary, tertiary and quaternary. The primary level of organization has a sequence of amino acids, secondary shows folding of these chains, tertiary depicts the three dimensional folding while quaternary has two or more polypeptide chains folded to give these dimensional structures.

5) Functional properties of proteins are related to their functions in biosystems. In foods proteins functions as gelating, emulsifying and foaming agents which is due to their water binding, hydrolytic and other properties.

6) **Enzymes** are protein molecules functioning as catalysts in biochemical reaction
Coenzyme is the non-protein part of enzymes which could be an organic molecules or a metal.
Cofactor is the metal as non-protein part of an enzyme.
Prosthetic group is the non-protein part of enzyme tightly bound to it.

7) Immobilized enzymes are the enzymes which are separated after catalyzing a biochemical reaction for reuse. The enzyme is absorbed on a solid support called the matrix. An example of immobilized enzyme is *glucose isomerase* which is responsible for high fructose syrup during treatment of corn syrup.

7.13 SOME USEFUL BOOKS

Lee A. Frank (1983). *Basic Food Chemistry*, The AVI publishing Company Inc.

John deMan (1980). *Principles of Food Chemistry*, The AVI publishing Company Inc.

Swaminathan M. (1999). *Essentials of Food and Nutrition*, vol. I The Bangalore printing and publishing Co. Ltd., Bangalore

Bennion Marion (1980). *The Science of Food*, John Wiley and sons

Manay N. Shakuntala and M. Shadaksharaswamy (1987). *Foods: Facts and Principles*, Wiley Eeastern Ltd.

Mudambi R. Sumati and Rao Shalini (1985). *Food Science*, Wiley Eastern Ltd.