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## **UNIT-4 PROTEINS**

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### **4.1 INTRODUCTION**

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Like carbohydrates and fats, proteins are essential to us in an incredible variety of different ways. Proteins are complex macromolecules, which constitute 50% or more of the dry weight of living cells. Muscle, ligament, cartilage, skin, hair etc. are mainly protein material. What are proteins? What is their composition, properties and functional applications in our food? These issues are discussed in this unit. The detailed structure and properties of proteins and amino acids are discussed in the Nutritional Biochemistry course, Block 1, unit 1. The functions, particularly the biological functions of proteins are dealt with in the Advance

Nutrition Course, block 1, unit 4. We suggest you to look at the structure, properties and functions of protein there before you move on to this unit. You will find a comprehensive review on the functionality of proteins in this unit, which as a food scientist, you will find quite useful.

### **Objectives**

After studying this unit, you will be able to:

- discuss the composition and classification of proteins,
- enumerate the properties of amino acids and proteins, and
- understand the applications of protein concentrates, isolates and hydrolysates.

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## **4.2 PROTEINS – CLASSIFICATION, COMPOSITION AND BIOLOGICAL FUNCTIONS**

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Proteins, as you may already know, are made up of carbon, hydrogen, nitrogen, oxygen and usually sulphur. Proteins are built up of a large number of amino acid molecules inter linked by peptide bonds as illustrated in figure 4.1. All the amino acids have a trivial or common name, many a times is related to the source from which they have been first isolated. For example:

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<b>Trivial Name of Protein</b>	<b>Source</b>
Asparagine	Asparagus
Glutamine	Wheat gluten
Tyrosine	Cheese ( <i>Tyros</i> is the <i>Greek word for cheese</i> )
Glycine- sweet taste	( <i>Glykos</i> is the <i>Greek word for sweet</i> )

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Amino acids are twenty in number, and you have read in the Nutritional Biochemistry Course that they are classified according to the nature of the side chain (R group). We shall not go into the classification and composition of amino acids here. This has been discussed in details in Unit 2 of the Nutritional Biochemistry Course. Look up the relevant section before you proceed further.

Figure 4.1: Peptide bond formation between two amino acids with the elimination of water

Let us look at the classification, composition and biological functions of proteins next. We start with the classification.

#### 4.2.1 Classification of Proteins

You may recall reading earlier about the classification of proteins in the Nutritional Biochemistry Course, Unit 2 and also in the Advance Nutrition Course, Unit 4. Let us review the classification here again. Box 4.1 summarizes the classification of proteins.

##### Box 4.1: Classification of Proteins

Proteins can be classified based on:

Shape and size	Functional properties	Solubility and physical properties
<ul style="list-style-type: none"> <li>Fibrous proteins for example: keratin in hair, actin and myosin in muscles, and collagen.</li> <li>Globular proteins, for example enzymes and antibodies.</li> </ul>	<ul style="list-style-type: none"> <li>Immunoproteins such as C-reactive protein, Opsonin, Immunoglobulins,</li> <li>Contractile</li> <li>respiratory,</li> <li>structural,</li> <li>enzymatic,</li> <li>hormonal</li> <li>carrier proteins</li> </ul>	<ul style="list-style-type: none"> <li>Simple proteins</li> <li>Conjugated proteins</li> <li>Derived proteins</li> </ul>

As can be seen in box 4.1, proteins can be classified based on:

- I *Shape and size*: fibrous proteins and globular proteins. Fibrous proteins play structural roles in organisms. Globular proteins consist of long chains of amino acids folded up into complex shapes.
- II *Functional properties*: Immuno, contractile, respiratory, structural, enzymatic, hormonal and carrier proteins.

III *Solubility and physical properties*: Simple, conjugated and derived proteins. Table 4.1 enumerates this classification.

**Table 4.1 Classification of proteins based on the solubility and physical properties**

Simple Proteins	Conjugated Proteins	Derived Proteins
<p>Simple proteins are those which are made of amino acid units only joined by peptide bond. Upon hydrolysis they yield a mixture of amino acids and nothing else.</p> <p><i>Examples:</i>  <b>Albumins:</b> Egg albumin, serum albumin, Lactalbumin  <b>Globulin:</b> Tissue globulin, serum globulin.  <b>Gliadins:</b> Wheat gliadin, hordein (barley) etc.  <b>Albuminoids:</b> Keratin of hairs, skin, egg shell and bones, elastin, Collagen of tendons, ligaments and bones.  <b>Histones:</b> globin of haemoglobin.  <b>Protamine:</b> Salmine, the spermatozoa of salmon fish.</p>	<p>Conjugated proteins are composed of simple proteins combined with a non-proteinous substance. The non-proteinous substance is called prosthetic group or cofactor.</p> <p><i>Examples:</i>  <b>Chromoproteins:</b> Haemoglobin, in which the prosthetic group is iron.  <b>Phosphoproteins:</b> casein in milk, in which prosthetic group is phosphoric acid, vitellin in egg yolk.  <b>Lipoproteins:</b> HDL (high density lipoprotein), LDL (low density lipoprotein) and VLDL (very low density lipoproteins), the prosthetic group is lipid  <b>Glycoprotein:</b> Ovomuroid of egg white.  <b>Nucleoproteins:</b> ribosomes and viruses.  <b>Metalloproteins:</b> alcohol dehydrogenase, a Zn containing enzyme.  <b>Mucoproteins:</b> Follicle stimulating hormone, <math>\beta</math>-ovomuroid.</p>	<p>These are not naturally occurring proteins and are obtained from simple proteins by the action of enzymes and chemical agents, heat, mechanical shaking, UV or X-Rays.</p> <p><i>Examples:</i>  Primary, Myosin, Fibrin, Secondary, Peptones, peptides, proteoses etc.</p>

Along with the classification, let us also quickly recapitulate the functions of proteins in our body. You may recall studying the functions of proteins in the Advance Nutrition course, block 1, unit 5. The next section summarizes the biological functions of proteins.

#### 4.2.1 Biological Functions of Proteins

Proteins have roles in the structural and functional aspects of the cells and organelles. Proteins may be classified according to the following plan:

*a. Structural elements:* Structural proteins are fibrous proteins. The most familiar of the fibrous proteins are probably the *keratins*, which form the protective covering of all land vertebrates: skin, fur, hair, wool, claws, nails, hooves, horns, scales, beaks and feathers. Equally widespread, if less visible, are the *actin* and *myosin* proteins of muscle tissue. Structural proteins are very important for support. *Collagen* and *elastin* provide a fibrous framework in animal connective tissues, such as tendons and ligaments.

*b. Defensive Proteins:* These proteins protect against diseases. Antibodies are the example of defensive proteins. These combat bacteria and viruses. Also, immunoglobulins, as you are already aware, provide defense to body against invading organisms and infections.

*c. Contractile Proteins:* These proteins participate in contractile processes such as *muscle proteins* as well as those found in other cells and tissues. In the latter, these proteins participate in localized contractile events in the cytoplasm, in motile activity, and in cell aggregation phenomena. The examples of contractile proteins include *actin*, *myosin*, *myoglobin*, *troponin* etc. Actin and myosin are responsible for the movement of muscles. Contractile proteins are responsible for the undulations of cilia and flagella, which propel many cells.

*d. Nutrient and Storage proteins:* These proteins store amino acids. *Ovalbumin* is the protein of egg white, used as an amino acid source for the developing embryo. *Casein*, the protein of milk, is the major source of amino acids for baby mammals. Plants store proteins in seeds.

*e. Transport proteins in plasma:* Transport proteins, embedded in lipid membranes, facilitate the import of nutrients into cells or the release of toxic products into the surrounding medium. Molecules which cannot move across the membrane by diffusion must cross the membrane with the help of transport proteins. As carriers of plasma, these bind to small molecules and ions and transport them throughout the body. Few common examples include:

- An iron-binding protein (*transferrin*) delivers ferrous ions to hemoglobin synthesizing loci.
- Activity of ions, such as calcium, can be controlled by the ratio of free to bound species.

- When hydrogen ions are bound, proteins act as *buffers* to minimize the change in pH.
- Since cells are impermeable to proteins, they also participate in determining the distribution of ions and hence electrical potential difference across the cell membrane.
- By virtue of osmotic activity, albumins mediate the distribution of body fluids between plasma and extracellular compartments

*f. Enzymatic Proteins:* The most varied and most highly specialized proteins are those with catalytic activity - *the enzymes*. Virtually all the chemical reactions of organic biomolecules in cells, you might be aware, are catalyzed by enzymes. More than 2,000 enzymes are known. Special class of enzymes fulfills a *mechanochemical role*, for example *actin, myosin and related proteins* of muscle structure are responsible for the conversion of chemical energy into mechanical work.

*g. Hormonal proteins:* Hormonal proteins coordinate the bodily activities. Various peptide and protein hormones (such as insulin and growth hormone) carry information that regulates cell permeability and cell metabolism.

***h. Receptor Proteins:*** These are built into the membrane of a nerve cell and they detect chemical signals released by other nerve cells. Receptor proteins are involved in the cell's response to chemical stimuli.

*i. Miscellaneous Functions:*

Besides the functions enumerated above certain other important miscellaneous functions of proteins are included herewith. These include:

- Source of energy: Constituent amino acids can be deaminated and metabolized to carbon dioxide and water.
- Toxic proteins (for humans - botulinum toxin, staphylococcal toxin, venom toxin; for microorganisms - antibiotics)
- Anti-nutritional factors (trypsin inhibitors).
- Many allergic reactions to food are also mediated by proteins, which result in the modification of defence mechanism of consumer due to the presence of proteinaceous antigens in foods that promote antibody synthesis.

- Intense sweeteners (Monellin).

#### 4.2.2 Composition of Proteins

We know that the amino acids are the building blocks of proteins. Amino acids are linked to one another by peptide bond formed by the elimination of a water molecule as illustrated in figure 4.1. Considering the long peptide chains and variation in the structure of twenty different amino acids, protein structure is divided into 4 different levels as illustrated in Table 4.1. The details related to the structure of each of these different proteins are included in the Nutritional Biochemistry Course (block 1, unit 1). Read the matter given there for comprehensive understanding of the protein structure.

**Table 4.1: Different levels of protein structure**

Primary structure	The primary structure of a protein consists of the order in which amino acids are bonded to one another by a peptide bond.
Secondary structure	The secondary structure of a protein involves the way that the chain of amino acid either twists or folds back upon itself to form alpha helical, beta sheet or a variety of other possible arrangements.
Tertiary structure	Secondary structure, in turn, folds back and bonds to itself in a three-dimensional manner.
Quaternary structure	When the protein consists of more than one chain, or the shape in which those separate chains bond together.

Besides the classification and composition of proteins, we would also spend some time recalling the food sources of proteins. We bet the protein food sources must be on your finger tips by now. Put them down and tally your responses with the sources given in the next section.

#### 4.2.3 Food Sources of Proteins

Food protein sources can be divided into 3 major categories:

- Protein of Animal Origin
- Protein of Plant Origin
- Single cell protein

Let us review these food sources one by one.

##### *a) Proteins of Animal Origin*

What are the foods of animal origin which can be classified as rich sources of proteins? You may already know, however, we have enumerated these sources herewith, for your perusal.

**1. Meat:** Skeletal or striated muscles are used for food purposes. Flesh of cattle, sheep and swine comprise most of the meat contents. Edible meat from these is designated as “Red Meat”, a term descriptive of colors of beef, lamb or pork, as opposed to the light and dark colors of poultry meat. The red color is primarily due to myoglobin.

A typical adult mammalian muscle stripped of all external fat contains about 18-22% proteins on wet weight basis. Muscle proteins can be categorized on the basis of their origin and solubility as sarcoplasmic, contractile (myofibrillar) or stroma (connective tissue) proteins.

**2. Milk:** A value of 3.5% protein is often considered as an average for milk. Milk protein has traditionally been divided into 2 classes – casein and whey protein. Casein is a heterogenous group of phosphoprotein, which can be precipitated from raw skimmed milk by acidification at pI.4.6 and 20<sup>0</sup>C. Proteins remaining in solution after casein precipitation are called ‘whey proteins’ (or milk serum proteins). Casein fraction consists of about 80% of total protein content, rest is whey protein. Whey fraction mainly consists of  $\beta$ -lactalbumin,  $\alpha$ -lactalbumin, immunoglobulins, bovine serum albumin etc.

**3. Eggs:** Roughly, the chicken egg consists of 11% shell, 31% yolk and 58% white. Liquid whole egg consists of 65% white and 35% yolk. The primary function of egg protein is to nourish the young chick and provide food. Yolk appears to be the initial source of food, while egg white seems to act as a protective barrier, prior to its eventual use as a source of protein. Fundamentally, the white and yolk differ in their composition.

- **Yolk:** Yolk contains about 50% solids, of which 2/3<sup>rd</sup> are lipids and proteins. On wet weight basis, egg yolk contains 31% fat, of which 1.3% is cholesterol.
- **White:** Essentially an aqueous solution containing about 12% proteins.

4. *Fish*: The edible portion is skeletal muscles of the body. Even though the skeletal muscles of different animals are basically similar, fish species used for food are far more numerous and diverse than the mammalian species.

Fish usually contains 40-60% edible flesh. Protein content of fresh water fish ranges from 13-25%. In the mid or lateral line of many fishes, there exists a layer of heavily pigmented reddish brown muscle that may contribute to 10% of total body muscle. This contains a high content of hemoprotein, which following the harvest, may catalyze oxidation of lipids and cause pronounced rancidity. The proteins may be classified as sarcoplasmic, myofibrillar or connective tissue protein.

5. *Shell Fish*: Information on shellfish is fragmentary and incomplete. In shell fish, the shell comprises of a large portion of live weight of the fish and thus their edible content is low. Eg. of shellfish are crabs, lobsters, prawns and muscle oysters etc.

Next, we shall study about the proteins of plant origin.

#### *b) Proteins of Plant Origin*

Vegetable proteins, cereal proteins, nuts and seed proteins come from the plant origin. Let us get to know more about these sources.

1. *Vegetable Proteins*: Fresh vegetables are not considered to be a very good source of proteins. On fresh weight basis, the average protein content of some common vegetables are carrots and lettuce – 1%, white potatoes, asparagus and green beans - 2% and fresh peas – 6%.

Although protein content of potatoes is only 2%, quality is considered to be good to excellent due to relatively high content of the amino acids – lysine and tryptophan. Outer layers, the so called “cortex” of tubers contain most of the proteins. These layers also have a much higher content of essential amino acids than do the inner layers. The outer layer proteins can be increased by selective plant breeding.

2. *Cereal Proteins*: Cereal grains, properly ripened and dried for optimum storage stability, have protein content ranging from 6-20%. Proteins are found in various

morphological tissues of different grains. In the milling of grain (eg. wheat), the endosperm is essentially separated from the bran and germ and then pulverized to produce flour, which is used as food. Endosperm proteins apparently act as a structural component and also as food reserves for the growing seedlings. Much of the endosperm storage proteins in kernel of several cereal proteins are located in the sub-cellular granules or organelles known as 'protein bodies' (except in wheat kernel).

Bran or seed coat protein provides structure and protection to kernel. Since bran is so poorly digested by humans and the proteins are difficult to separate, most of the material is used for animal feed.

**3. Seed Proteins:** Although a large number of plants produce seeds having protein contents in excess of 15%, only a few are utilized for food, eg. soybean, cotton seeds, peas, peanuts and beans. Proteins of seeds are largely concentrated in protein bodies. These bodies, which have more than 90% proteins accounts for 70% of the total proteins in case of soybeans.

Proteins comprise a significant portion of food reserves, which is so important during germination. Proteins of most seeds (excluding cereals) are globulins, which are soluble in water or dilute salt solutions.

**4. Nuts:** Nuts are excellent sources of proteins. Examples of nuts include cashew nuts, almond nuts , hazel nuts , coconuts, walnuts, brazil nuts ,cashew nuts, pistachio nuts etc. Some nuts like almonds contain complete proteins.Those nuts that do not contain complete proteins can be extremely useful sources of proteins if they are eaten in combination with other protein foods, or with milk or cheese, or with vegetables.

Having studied about the proteins of plant origin we shall move on to study about proteins obtained from the microbial origin.

### *c) Single Cell Proteins (SCP)*

You may have heard of SCP. What is a single cell protein? Let's find out.

The term SCP was coined by Prof. Carroll Wilson (MIT) in 1966. It means the proteins obtained from microbial sources, i.e. algae, fungi, bacteria, yeast etc. The proteins are isolated from microorganisms. Some of the advantages of selecting microorganisms as a source of protein are as follows:

- a. High yield of proteins on dry weight basis.
- b. Nutritional requirement is cost effective.
- c. Less area is required for the installation of plant for the production of proteins as compared to the classical sources.
- d. The plant may be designed in such a way that the processing can be done on a continuous basis instead of batch to batch basis.

For single cell proteins, bacteria, yeast, fungi and algae may be used. Each of them has their own advantages and disadvantages. These have been highlighted in Table 4.2. Another interesting aspect which you would surely like to know about is how these single cell proteins are manufactured. You will find information regarding the process in Box 4.2 herewith. This is some additional information for your knowledge.

**Table 4.2: Advantages and disadvantages of using microorganisms as a source of protein**

Organisms	Advantages	Disadvantages
Bacteria	<ul style="list-style-type: none"> <li>• High yield of protein (60-80%)</li> <li>• Can be grown on paraffin, cellulose waste and molasses.</li> <li>• Growth rate is fast.</li> </ul>	<ul style="list-style-type: none"> <li>• If the bacterial strain is very small in size and of low density, separation from the culture media is difficult.</li> <li>• Bacteria have a high nucleic acid content (&gt;15%) and may come over along with proteins, which is not acceptable and not required as it can cause hypertension, arthritis etc.</li> <li>• Uric acid, the final product of purine metabolism, may lead to gout.</li> </ul>
Yeast	<ul style="list-style-type: none"> <li>• Large size, hence separation from the culture medium is easy.</li> <li>• As the pH of the growth is towards acidic side, high amount of lysine is produced in the proteins, hence protein is more acceptable and of higher biological</li> </ul>	<ul style="list-style-type: none"> <li>• Less protein yield (45-60%)</li> <li>• Growth rate is low (1-3 h)</li> <li>• High nucleic acid content leading to the formation of uric acid.</li> </ul>

	value.	
Fungi	<ul style="list-style-type: none"> <li>• Easy to harvest from culture medium.</li> <li>• Texture of the fungi improves the functional properties of proteins.</li> </ul>	<ul style="list-style-type: none"> <li>• Less protein yield (5-27%).</li> <li>• Low growth rate.</li> <li>• Poor acceptability of proteins.</li> </ul>
Algae	<ul style="list-style-type: none"> <li>• Produces proteins which have almost all the Essential Amino acids.</li> <li>• Rich in tyrosine and serine, low in sulphur containing amino acids.</li> </ul>	<ul style="list-style-type: none"> <li>• If the more than 100 gm of proteins are consumed, it may cause nausea, vomiting, abdominal pain etc. because of the cellulosic cell wall, which is not digestible in human subjects.</li> </ul>

**Check Your Progress Exercise 1**

1. How can proteins be classified? Give suitable examples.

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2. Mention any two important functions of proteins in biological systems.

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3. What are the 3 categories in which food protein sources can be divided into?

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4. What is a single cell protein? Also, discuss some of the advantages of selecting microorganisms as a source of protein.

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5. Fill in the blanks:

- a) The proteins made up of only amino acids are----- while those containing non-proteinaceous prosthetic group are-----
- b) A Zn-containing enzyme, which is a conjugated protein is-----

- c) A class of enzymes involving the conversion of chemical energy into mechanical work fulfills a -----
- d) Proteins that act as structural elements are-----
- e) -----provide defence to the body against invading organisms and infections.
- f) A protein consisting of more than one chain, or the shape in which those separate chains bond together is-----.
- g) Meat muscle proteins can be categorized as----- .
- h) Endosperm proteins act as ----- while bran proteins provides----- .

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### 4.3 FUNCTIONAL PROPERTIES OF PROTEINS

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It may be clear by now that functionality (as implied to food ingredients) refers to *'any property aside from the nutritional attributes that influence usefulness of ingredients in the food'*. Most of the functional properties affect the sensory characteristics (especially textural attributes) of foods, but also can play a major role in the physical behavior of food and food ingredients during their preparation.

Thus, *functional properties of proteins are those physico-chemical properties that enable the proteins to contribute to the desirable characteristics of the food*. You may recall reading about these physical, chemical properties of proteins in the Nutritional Biochemistry Course, in Unit 2. For your convenience, we have summarized the physical properties here.

So far, we have learnt that proteins may be added as components of foods for functional, nutritional or economic benefits. Potential functional benefits include *emulsification* and *stabilization*, increased *viscosity*, improved *appearance, taste* or *texture, form foams* and *gels* and *binding of fat* or water. These functional properties allow the technologist to modify flow properties, emulsify, form gels and foams or bind water and fat. Nutritional benefits include lowering the caloric contents of foods, increasing the protein level and balancing

the amino acid profile. Of course, the economic or cost considerations are also important while using protein as an ingredient. An example of an economic benefit would be increased yield of the product from the use of a protein additive. It is also important to know that the type of protein (animal or plant origin) and the structure of protein will determine its functional properties. Three groups of functional properties of proteins have been identified. These include:

a) *Hydration properties* (dependent on protein-water interactions), which include properties like swelling, adhesion, dispersibility, solubility and viscosity.

b) *Properties related to protein-protein interactions*, which involves the processes of precipitation, gelation and formation of other structures (like protein doughs and fibers).

c) *Surface properties*, which relates primarily to surface tension, emulsification and foaming characteristics of proteins.

As you can see, these three groups are not totally independent. For example, gelation involves not only protein-protein interactions but also protein-water interactions. Also viscosity and solubility are both dependent on protein-water as well as protein-protein interactions. Let us learn about these functional properties, beginning with hydration properties.

#### *a. Hydration Properties*

General conformation of individual proteins in solution is largely dependent on the interaction with water. The progressive hydration of proteins starting from the dry state, the sequential steps are shown in the figure 4.2, are postulated:

Many functional properties of protein preparations are related to this progressive hydration. Water absorption (water uptake, affinity or binding), swelling, wettability, water holding capacity (water retention) and also cohesion and adhesion are related to the first four steps (figure 4.2), whereas dispersibility and viscosity (or thickening power) involves 5th step also. Final state of protein, either soluble or insoluble (partially or totally) is also related to important functional properties, such as solubility or instant solubility (in which all five steps take place rapidly). Gelation implies 'the formation of well-hydrated insoluble mass with specific protein-protein interactions'. Finally, surface

properties, such as emulsification and foaming, necessitates a high degree of protein hydration and dispersion in addition to their characteristics.

Let us learn more about the hydration properties, which include solubility, viscosity etc.

### *Precipitation/Solubility of Proteins*

Most of the functional properties are dependent on the degree to which the proteins are soluble. The solubility behaviour provides a good index of potential application of proteins. This is so because the degree of insolubility is probably the most practical measure of protein denaturation and aggregation and because proteins that initially exist in a denatured, partially aggregated state, often have an impaired ability to participate effectively in gelation, emulsification and foaming.

Note, in general, proteins, which are highly soluble, may be used in applications where emulsification, whipping and film formation are important whereas low solubility may be desired in applications with high protein levels and when limited emulsification or protein-protein interactions are needed.

Solubility of proteins is markedly and irreversibly reduced when heating is involved. However, heat treatments may be unavoidable to achieve other objectives (microbial inactivation, removal of off-flavor, removal of water and others).

Assumption that the proteins must have a high initial solubility as a prerequisite for other functional properties is not always correct. It has already been noted that water absorption of a protein ingredient sometimes can be improved by prior denaturation and partial insolubilization. This is in agreement with the fact that the formation of emulsions, foams and gels can involve various degrees of protein unfolding, aggregation and insolubilization.

On the other hand, whey proteins and some other proteins must have reasonably high initial solubility, if they are to function well in the emulsification, foams and gels. Soluble caseinates have better thickening

and emulsifying properties than isoelectric casein (less soluble). Perhaps the main advantage of insolubility is that it permits rapid and extensive dispersion of protein molecules and particles which leads to a finely dispersed colloidal system with homogeneous macroscopic structure and smooth texture. Also, initial solubility facilitates protein diffusion to air/water and oil/water interface, thus improving their surface activity.

#### *Viscosity, Gelation and Texturization*

**Viscosity** reflects resistance to flow. The main single factor influencing the viscosity behavior of protein fluids is the apparent diameter of the dispersed molecule, which is dependent on the following parameters :

- a. Intrinsic character of protein molecule (molar mass, size, volume, structure and asymmetry, electric charges and ease of deformation), environmental factors such as pH, ionic strength and temperature, can modify these characteristics through unfolding;
- b. Protein solvent interaction which influence swelling, solubility and hydrodynamic sphere surrounding the molecule, and
- c. Protein-protein interactions which determine the aggregates. Protein ingredients are generally used at a high concentration at which protein-protein interactions predominate.

Viscosity and consistency of protein systems are the important functional properties in fluid foods, such as beverages, soups, sauces and creams. Correlation between viscosity and solubility is not simple. Insoluble heat denatured protein powders do not develop high viscosity when placed in aqueous medium. Highly soluble protein powders with low water absorption and swelling capacities (whey proteins) also exhibit low viscosity at neutral or IpH. Soluble protein powders with high initial water absorption capacities (sodium caseinates and some soy protein preparations) develop a high viscosity. Thus, for many proteins, a positive correlation is observed between water absorption and viscosity.

**Gelation** refers to the process where denatured molecules aggregate to form an ordered protein network. Proteins can form a well-ordered gel matrix by

balancing protein-protein and protein-solvent interactions in food products. These gel matrices can hold other food ingredients in producing food products, like gelatin, yoghurt, comminuted meat products, tofu and bread doughs.

Gelation is an important functional property of several proteins that plays a major role in preparation of many foods, including various dairy products, coagulated egg whites, gelatin gels, various heated, comminuted meat or fish products, soy bean protein gels, vegetable proteins texturized by extrusion or spinning and bread doughs. Protein gelation is utilized not only for the formation of viscoelastic gels but also for improved water and fat absorption, thickening, particle binding (adhesion) and emulsion or foam stabilizing effect.

While studying about gelation, it is also important to differentiate it from other related phenomena in which the degree of dispersion of protein solution is decreased. For eg.

- **Association:** refers to changes occurring at subunit or molecular level.
- **Polymerization or aggregation:** involves formation of large complexes.
- **Precipitation:** includes all aggregation reactions with total loss of solubility.
- **Flocculation:** random aggregation reaction in the absence of denaturation; often occurs because of suppression of electrostatic repulsion between the chains.
- **Coagulation:** random aggregation with denaturation and aggregation reactions where protein-protein interactions predominate over protein-solvent interactions and forms a coarse coagulum.

Next, let's look at the texture function of proteins.

### *Texturization*

Proteins constitute the basis of structures and texture in several foods, whether these come from living tissue (myofibrils in meat or fish) or from

fabricated substances (bread dough and crumb, soy or gelatin gels, cheese, curds, sausage, meat emulsion etc).

Also, there are a number of texturization processes that begin with soluble vegetable or milk proteins and that lead to film or fiber like products with chewiness and good water holding capacity and that have the ability to retain these properties during subsequent hydration and heat treatment. These texturized proteins are often used as meat substitutes and/or extenders. Also, some texturization processes are done for the purpose of retexturization or reforming animal proteins such as beef or poultry meat. Known physicochemical basis of some of these texturization processes are presented below:

**1. Thermal Coagulation and Film Formation:** Concentrated soy proteins can be thermally coagulated on a flat metallic surface, such as that of a drum dryer. Resulting thin, hydrated films can be folded, pressed together and cut.

**2. Fiber formation:** Fiber spinning of vegetable (especially soy) and milk proteins bears many similarities to the spinning of synthetic textile fibers. It is usually necessary to start from isolates containing 90% protein or more. Four to five successive operations are necessary and can be done continuously.

**3. Thermoplastic Extrusion:** This is a major technique used for vegetable proteins at present. Thermoplastic extrusion leads to dry fibrous and porous granules or chunks, (rather than fibers) which upon rehydration, possess a chewy texture. Starting material need not to be protein isolates, thus less costly protein concentrates or flours (containing 45-70% protein) can be used. Casein or gluten can be added as such. Addition of small amount of starch or amylose improves the final texture, but a lipid content above 5-10% is detrimental. Addition of 3% sodium chloride or calcium chloride also firms the texture. Good texturization by this process requires proteins with appropriate initial solubility, high molecular weight and development of proper plasticizing and viscosity properties of protein-polysaccharide mixture within the dye.

After texturization, we move on to *properties related to protein-protein interactions*.

*b. Properties related to protein-protein interactions*

Properties related to protein-protein interactions include dough formation, one of the important functional properties of proteins. Let us get to know about dough formation.

*Dough Formation*

You already know that gluten is the protein found in wheat. A unique property of gluten proteins of wheat grain endosperm (and to a lesser extent of rye and barley grains) is their ability to form a strongly cohesive and viscoelastic mass or dough, when mixed and kneaded in presence of water at ambient temperature. In addition to glens (gliadin and glutenins), wheat flour contains starch granules, pentosans, polar and non polar lipids and soluble proteins, all of which contribute to the formation of dough network and/or the final texture of bread.

Composition and large size of gliadins and glutenins explain much of the behaviour of gluten. Due to their low content of ionizable amino acids, the gluten proteins are poorly soluble in neutral aqueous solutions. Rich in glutamine (>33% by wt.) and in hydroxy amino acids, they are prone to hydrogen bonding which accounts largely for water absorbing capacity and for the cohesion and adhesion properties of gluten. Latter properties also derive in part from the presence of many apolar amino acids and the resulting hydrophobic interactions that contribute to protein aggregation and binding of lipids and glycoproteins. Finally, the ability of forming numerous -S-S- cross linkages accounts for the ease with which these proteins interlink tenaciously in dough.

We have been kneading doughs for long, without understanding the process going on within. What are the changes occurring in gluten proteins during dough formation? Box 4.3 presents a detailed discussion on this topic. You will find this information interesting.

***Box 4.3 : Changes In Gluten Proteins During Dough Formation***

Initially, gluten is formed when flour and water are mixed together. The proteins in the flour, glutenin and gliadin cross link, using water as a vehicle to form gluten. Enhancing this gluten structure is important relative to developing a gas retaining structure in the chapati/bread. When the hydrated bread flour is mixed and kneaded, the gluten proteins orient themselves aligns and partially unfolded. This enhances hydrophobic interaction and formation of disulphide bridges through -S-S- interchange reactions. A 3-dimensional viscoelastic protein network is established, as the initial gluten particles transform into this membrane (film), thus serving to entrap starch granules and other flour components. Cleavage of disulphide bridges by reducing agents such as cysteine, destroys the cohesive structure of hydrated gluten and bread dough ; the addition of agents such as bromates, increase toughness and elasticity. "Strong" flours from certain wheat varieties require long mixing time and give very cohesive dough. "Weak flours" are less effective and gluten network breaks down when the energy or duration of mixing exceeds a certain level, probably because of -S-S- bonds are ruptured (especially in absence of air). Dough strength appears to be related to a large content of high molecular weight glutenins including totally insoluble "residue proteins". From experiments with "reconstituted" wheat flours of varying gliadin and glutenin ratios, it can be postulated that the glutenins are responsible for the elasticity, cohesiveness and mixing tolerance of dough whereas gliadins facilitate fluidity, extensibility and expansion of the dough, thus contributing to a larger bread loaf volume. A proper balance of the proteins is essential for bread making. Excessive cohesion (glutenins) inhibit the expansion of trapped CO<sub>2</sub> bubbles during fermentation, the rise of the dough and the subsequent presence of open air cells in the bread crumb. Excessive extensibility (gliadins) results in gluten films that are weak and permeable; thus retention of CO<sub>2</sub> is poor and dough collapse may occur.

### *C. Surface properties of proteins*

The surface properties relates primarily to surface tension, emulsification and foaming characteristics of proteins, which are discussed herewith:

#### *Emulsifying properties of proteins*

Proteins are the surface-active substances, which are extensively used in the food industry as emulsifiers to manufacture products such as desserts, spreads or whipped cream.

*Protein Stabilized Food Emulsions:* Many food products are emulsions (eg. milk cream, ice creams, cream, butter etc.) and protein constituents often play a major role in the stabilization of these colloidal systems. In the fresh milk, soluble proteins are immunoglobulins. Homogenization of milk increases emulsion stability because it reduces the size of fat globules and because newly formed casein submicelles displace the immunoglobulins and adsorb to fat globules.

The proteins stabilize emulsions and contribute physical and rheological properties, like thickness, viscosity, elasticity and rigidity that determine resistance to droplet coalescence. Ionization of amino acid side chains may also take place depending on the pH and this provides electrostatic repulsive force that favors emulsion stability. This functional property is important in the formation of many common food products, such as salad dressings and sausages.

Proteins are generally poor stabilizers of water/oil (w/o) emulsion. You will be reading about emulsions in details in unit 7 of this course. This may be attributable to the predominantly hydrophobic nature of most proteins, causing the bulk of an adsorbed protein molecule to reside on the water side of interface.

Proteins can bind to water, lipids, volatile flavours and other substances and possess important functional properties. Let's get to know them.

#### *Foaming Properties, Binding of Flavour and Other Substances*

To understand the foaming properties of proteins, we need to know some basic aspects of foam foods. **Foam foods** are usually colloidal dispersion of gas bubbles in a continuous liquid or semisolid phase that contains a soluble surfactant. (Surfactant is a chemical compound that acts as a surface modifier which reduces the surface tension of the liquid). A large variety of food foams produced with proteins, exist with widely differing textures, such as cakes, whipped creams and toppings, ice creams etc. In many cases, gas is air

(occasionally CO<sub>2</sub>) and the continuous phase is an aqueous or suspension containing proteins. You will read more about foams in unit 7.

Next, what are the properties basic to proteins, to be a good foaming agent? A protein must:

- be able to rapidly absorb at the air-water interface during whipping,
- undergo rapid arrangement and rearrangement at the interface, and
- form cohesive viscoelastic film.

### *Flavour Binding*

Some protein preparations, although acceptable from a functional and nutritional stand point, necessitate a deodorizing step to remove the bound off-flavors. Various substances, such as aldehydes, ketones, alcohols and oxidized fatty acids may cause beany or rancid odours and bitter or astringent taste. When bound to proteins or to other constituents, these substances are released and become perceptible after cooking and/or mastication. Some are so strongly bound that even steam or solvent extraction do not remove them.

Quite different from the problem of off-flavour removal, it may be useful to use proteins as carriers for desirable flavours. It is of interest to impart a meat flavour to texturized vegetable proteins. Ideally, all of the volatile constituents of desirable flavour must remain bound during storage, possibly also due to processing and then be released quickly and totally in the mouth without distortion. Problems mentioned above can be solved through investigation of the mechanism by which volatile compounds bound to proteins. Let's find out.

### *Interactions between Volatile Substances and Proteins*

Flavour binding may involve adsorption at the surface of food or penetration to the food interiority by diffusion (absorption). By adsorption we mean, a surface phenomenon that involves adhesion of the molecules of gases, dissolved substances, or liquids in more or less concentrated form to the surface of solids/liquids with which they are in contact.

Flavour binding by adsorption implies the mechanism of physical or chemical adsorption and hydrophobic interaction. Polar compounds, such as, alcohols are

bound via hydrogen binding but hydrophobic interactions with nonpolar amino acids predominate in the binding of low molecular weight volatile compounds.

In some cases, volatile compounds bind to proteins via covalent linkages and the process is usually irreversible. Irreversible fixation is more likely to occur with the volatiles of high molecular weight.

### ***Binding of Other Compounds***

In addition to water, lipids and volatile flavours, food proteins can bind a number of other substances through weak interactions or through covalent bonds, depending upon their chemical structure. Eg. pigments, synthetic dyes (which may be used for analytical determination of proteins) and substances with mutagenic, sensitizing biological activity. Such binding may result both in enhanced toxicity or detoxification and in some cases; the nutritional values can be adversely affected.

### **Check Your Progress Exercise 2**

1. Fill in the blanks:
  - a) In wheat proteins, gliadins are responsible for -----whereas glutenin imparts ----- to the dough.
  - b) The three groups of functional properties are-----, -----, and -----.
  - c) ----- is one of the factors affecting protein solubility.
  - d) Colloidal dispersion of gas bubbles in a continuous liquid or semisolid phase that contains a soluble surfactant are ----- foods.
  - e) Highly soluble proteins are used in three applications such as -----, -----, and -----.
2. What do you understand by the term functional properties? List the three functional properties of proteins.

-----  
-----  
-----  
-----  
-----  
-----

3. Following are the functional properties of proteins. Comment on the role of these in food preparation.

a) Gelation

-----  
-----  
-----

b) Texturization

-----  
-----  
-----

c) Stabilizer

-----  
-----  
-----

Having learnt about the functional properties of proteins, we now focus on the application of proteins. You may have heard about protein concentrates, isolates etc. What are these products? The next section unfolds the discovery, uses of these products.

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#### **4.4 PROTEIN CONCENTRATES, ISOLATES AND HYDROLYSATES AND THEIR APPLICATIONS**

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The first question that may come to your mind is why do we need to concentrate or isolate a protein from a product? The major purpose of the preparation of concentrates and isolates from a protein source is to increase the concentration of proteins by the removal of non protein ingredients form the source, so that the smaller amounts can be used in the formulation to impart nutritional as well as functional properties.

The methods utilized for the removal of non-protein ingredients should be such that it should not affect the nutritional and functional properties of the protein to a great extent. Most of the work has been done on the preparation of soy protein

isolates and concentrates and whey protein concentrates. Let's learn from these discoveries.

#### **4.4.1 Protein Concentrates**

In this section, we will learn about the soy protein and whey protein concentrates. What are they and what are their uses? Let's get to know them.

##### *A. Soy Protein Concentrates*

The Association of American Feed Control Officials, Inc. (AAFCO) specifies soy protein concentrates as follows:

*"Soy Protein Concentrate is prepared from high quality, sound, clean, dehulled soybean seeds by removing most of the oil and water soluble non-protein constituents and must contain not less than 70% protein on a moisture free basis."*

Edible soybean protein concentrates are relatively new products. Their availability as commercial products dates from 1959. In the last 30 years or so, these versatile products have become important ingredients, well accepted by many food industries. In many applications, they simply replace soy flours. In others, they have specific functions, which cannot be performed by soy flours.

Historically, the need for the development of soybean protein concentrates stemmed primarily from two considerations: to increase protein concentration and to improve flavour. The products containing about 70% protein are prepared from defatted meal by selective extraction of the soluble carbohydrates (sugars). Extraction with aqueous alcohol is the most common process, but other methods of production are also available. The concentrates are essentially bland.

Soybean protein concentrates normally cost 2 to 2.5 times more than defatted soy flour. Considering the relative protein contents of these two products, the cost per unit weight of protein is about 80% higher in the concentrate.

The starting material for the production of soy protein concentrates is dehulled, defatted soybean meal with high protein solubility (white flakes). The concentration of protein is increased by removing most of the soluble non-protein

constituents. These constituents are primarily soluble carbohydrates (mono, di and oligosaccharides), but also some low molecular weight nitrogenous substances and minerals. Since some low molecular weight proteins are also extracted along with the sugars, the amino acid composition of the concentrates may differ slightly from that of the original flour.

Now let us look at the uses of this product.

#### *Utilization of Soy Protein Concentrate*

Soy protein concentrates are used in food products for their nutritional characteristics or for their functional properties or for both. Nutritionally, the attractive features of concentrates include their high protein content, the near-absence of anti-tryptic and other anti-nutritional factors, the absence of flatulence and the substantial "dietary fibre" content. The nutritional value of the protein in the concentrates of different types, expressed as Protein Efficiency Ratio (PER), is slightly lower than that of soy flour protein. This is probably due to the slight fractionation effect of the extraction process, mentioned above. The most important functional characteristics of soy protein concentrates are water-binding (water adsorption) capacity, fat binding capacity and emulsification properties. The use of soy protein concentrates in different applications in food industry is highlighted herewith.

##### *a. Bakery products*

Unless higher protein fortification levels are necessary, there is no special reason for using soy protein concentrates in bakery products. Nutritionally and functionally, soy flours do the same job, more economically.

##### *b. Meat products*

This area probably represents the most important application of soy protein concentrates (SPC) in the food industry. SPC is used mostly in comminuted meat, poultry and fish products (patties, emulsion type sausages, fish sticks etc.) to increase water and fat retention. The nutritional contribution of soy protein in low-meat, high-fat, low-cost products may also be significant. Typical usage levels, on moisture-free basis, are 5-10% in patties, 2-8% in chilli, 2-12% in meatballs, 3.5% maximum in sausages and 5-10% in fish sticks.

##### *c. Other uses*

Soybean protein concentrates have been used as stabilized dispersions in milk-like beverages and simulated dairy products, such as sour cream analog.

After soy protein concentrates we shall study about the whey protein concentrates.

### *B. Whey Protein Concentrates*

You already know that whey is the residual liquid substance that is obtained by separating the coagulum from milk during cheesemaking. There are important components contained in whey, the most valued of which are the proteins which are highly regarded for their nutritional properties. The major whey proteins are  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin. These globular proteins offer the most diverse functional benefits and have the greatest potential when used in further processed foods.

What, then, is the whey protein concentrate?

Whey protein concentrates (WPC) are the products derived from whey from which the water, minerals and lactose have been removed. WPC is a white to light cream-colored product with a bland, clean flavor. It is manufactured by drying the material resulting from the removal of sufficient non-protein constituents from pasteurized whey so that the finished dry product contains 25% or more protein. The non-protein constituents are removed by physical separation techniques such as precipitation, filtration, or dialysis. WPC can be used in fluid, concentrate, or dry product form. Safe and suitable pH-adjusting ingredients may be used to adjust the acidity of WPC.

Whey protein concentrate (WPC) is a highly nutritious ingredient manufactured from fresh dairy whey and it is spray dried to provide an excellent source of low fat dairy protein. Useful properties, such as high solubility and water retention capacity, make it an ingredient of choice in a wide variety of functional and processed foods, beverages and health supplements. Whey protein concentrates can also contribute a high level of viscosity and structure to food formulations as well as a smooth texture. Generally, WPC with higher protein content have

improved functionality over those with lower protein content. Some of the basic functionalities of WPC are highlighted in Box 4.4.

**Box 4.4 Functional Properties of WPC**

<b>Functional property</b>	<b>Mode of action</b>	<b>Food system</b>
<b>WaterBinding/ Hydration</b>	Proteins can help reduce formula costs as the proteins hold additional water.	Meats, beverages, breads, cakes, sausages
<b>Gelation/Viscosity</b>	Protein-protein interactions produce matrix formations and setting	Salad dressings, soups, setting cheeses, baked goods, gravies, meats
<b>Emulsification</b>	Proteins stabilize fat emulsions	Sausages, soups, cakes, salad dressings, infant foods
<b>Foaming/Whipping</b>	Proteins form stable film. Foaming properties are best when the whey proteins are undenatured, not competing with other surfactants at the air/water interface.	Whipped toppings, chiffon cakes, desserts
<b>Browning/Flavour/ Aroma</b>	Proteins contribute to browning by reacting with lactose and other reducing sugars present in a formulation, providing colour to heated products.  WPC is bland tasting and contribute no foreign or off-flavours when used as an ingredient.	Confections, meats in microwave, sauces, breads, low-fat baked goods, soups, dairy products.

What are the uses of WPC?

Whey protein concentrates are used extensively in the manufacture of baked goods, where they increase the effectiveness of shortening through better dispersion and contribute to product browning, crust and flavor development. High viscosity whey protein concentrates are ideally suited to use in ice cream, processed cheese, sauces/slices and various fresh dairy and specialized nutritional applications. Whey proteins have also been designed with enhanced heat stability,

water retention and gelation for superior performance in beverages, infant formula, soups, sauces, frozen yogurt and ice cream.

WPC of 35 percent protein is commonly used as a replacement for skimmed milk, as well as a stabilizer and fat mimetic in yogurt, bakery mixes, dietetic foods, infant foods and confections. Its water-binding properties, fat-like mouth-feel and gelation properties are of particular benefit when used in these further processed products.

WPC of 50, 65 or 80 percent protein are especially suited for use in nutritional drinks, soups, bakery products, meat, dietetic foods, low-fat products and protein-fortified beverages. They are especially noted for their ability to completely dissolve in a wide range of pH conditions.

Defatted WPC powder containing 80 - 85 percent protein is an excellent alternate ingredient to use in certain applications, more notably as an economical egg-white replacer in whipped products such as meringues, modern ice-cream and toppings.

So far we have studied about the soy and whey protein concentrates. It is interesting to note that soy and whey protein also exist as isolates. What is the difference between concentrates and isolates? Read to find out.

#### **4.4.2 Protein Isolates**

Before we begin our discussion on protein isolates, let us first get to know how protein concentrates differ from isolates. Basically, the two differ in the process by which the proteins are produced, and the quality of the protein. Isolate is always higher quality protein. Do remember.

In this section, we will focus on soy protein isolates.

##### ***Soybean protein isolates:***

Soy protein isolates are the most pure and refined soy protein available. Isolated soybean proteins (ISP), or soybean protein isolates, are the most concentrated form of commercially available soybean protein products. They contain over 90%

protein, on a moisture free basis. Soy protein isolates have been known and produced for industrial purposes, mainly as adhesives for the paper coating industry, well before World War II. ISP's for food use, however, have been developed only in the early fifties.

The specification of the Association of American Feed Control Officials, Inc. (AAFCO) defines ISP as "*Major proteinaceous fraction of soybeans prepared from dehulled soybeans by removing the majority of non-protein components and must contain not less than 90% protein on a moisture-free basis.*"

The basic principles of ISP production are simple. Soybean protein isolates are obtained by selective solubilization of the protein (e.g. alkaline extraction), followed by purification of the extract and precipitation of the protein (e.g. by acidification to the isoelectric point). Isoelectric isolates are insoluble in water and have practically no functional features. They can be converted to sodium, potassium or calcium proteinates by dissolving isoelectric proteins in the appropriate base and spray-drying the solution. Sodium and potassium proteinates are water-soluble. They are used mainly for their functional properties, such as emulsification or foaming. One of the by-products of the protein isolation process, the insoluble residue, is also commercialized for its remarkable water absorption capacity and as a source of dietary fibre.

Since spray-drying is the common drying method in the production of ISP, the primary physical form of ISP in commerce, is that of fine powders. Structured forms, such as granules, spun fibres and other fibrous forms are made by further processing.

Being almost a pure protein, ISP can be made to be practically free of objectionable odour, flavour, colour, anti-nutritional factors and flatulence. Furthermore, the high protein concentration provides maximum formulation flexibility when ISPs are incorporated into food products. These and other advantages have been the source of highly optimistic forecasts regarding the widespread use of ISP. The various uses of ISP in food applications are discussed herewith.

### *Uses of Soy protein isolates*

The applications of ISP in various food products are enumerated herewith:

- *Meat products*

The major application of ISP in connection with meat and related products is based on the use of texturized ISP, in one form or another, to replace meat.

In emulsion type sausages, such as frankfurters and bologna, ISP and proteinates are used for their moisture and fat binding properties and as emulsion stabilizers. The use of ISP in these products permits reducing the proportion of expensive meat in the formulation, without reducing the protein content or sacrificing eating quality. Methods for incorporating soy protein products into whole muscle meat have been developed recently.

- *Seafood products*

The most important application in this category is the use of ISP in fish sausage and surimi based restructured fish products in Japan. Surimi is an extensively washed, minced fish flesh.

- *Cereal products*

ISP is sometimes used instead of, or in combination with isolates and soy flour, in the formulation of milk replacer mixtures in bakery products. ISP has been used for protein fortification of pasta and specialty bread. In these applications, the high protein content and blandness of ISP are clear advantages.

- *Dairy-type products*

Soybean protein isolates are used in non-dairy coffee whiteners, liquid whipped toppings, emulsified sour cream or cheese dressings, non-dairy frozen deserts etc. The basis for these applications is, demand for non-dairy (all-vegetarian, cholesterol-free, allergen-free) food products, as well as economy. Imitation cheeses have been produced from isolated soy proteins, with or without milk whey components. The types of cheeses which can be produced include soft, semi-soft, surface-cultured (imitation Camembert) and ripened hard cheeses.

- *Infant formulas*

Infant formulas, where milk solids have been replaced by soy products, are well established commercial products. ISP is the preferred soy ingredient, because of its blandness, absence of flatus-producing sugars and negligible fibre content. The principal market for these products are lactose-intolerant babies. However, soy

protein based dietetic formulas are finding increasing use in geriatric and post-operative feeding as well as in weight reduction programs.

- *Other uses*

Partially hydrolysed soy proteins possess good foam stabilization properties and can be used as whipping agents in combination with egg albumen or whole eggs in confectionary products and desserts.

Isolated soybean protein has been shown to be an effective spray-drying aid in fruit purees. In this application, it can replace maltodextrins, with the advantage of contributing protein to the final product. A nutritious "shake" base was produced by spray-drying ripe banana puree containing up to 20% ISP on dry matter basis.

Before we end, a final word on protein isolates. The cost of isolated soybean proteins is five to seven times higher than that of defatted soy flour. On an equal protein weight basis, the cost ratio of these two products is nearly 3:1. The main reasons for the added cost will become evident from the description of the manufacturing methods for ISP.

Now, we move on to the third concept, i.e. protein hydrolysates.

#### **4.4.3 Protein Hydrolysates**

What are protein hydrolysates? *Proteins that have been treated with enzymes to break them down into amino acids or shorter peptides are referred to as protein hydrolysates.*

Protein hydrolysates are valued for their superior nutritional qualities, including increased bioavailability and reduced antigenicity. Several enzymatic modifications of proteins / enzymes are known to occur in biological systems. Such modifications of proteins *in vitro* can be used to improve their functional properties. Only few of the enzymatic modifications of proteins are practical for modifying proteins for food use.

Hydrolysis of food proteins using proteases (trypsin, chymotrypsin, papain and thermolysin) alters their functional properties. Extensive hydrolysis by nonspecific proteases, such as papain, cause stabilization of even poorly soluble proteins. Such hydrolysates usually contain low molecular weight peptides of the

order 2-4 amino acids residues. Extensive hydrolysis damages several functional properties, such as gelation, foaming and emulsifying properties. These modified proteins are useful in liquid-type foods, such as soups and sauces, where solubility is a primary criterion and feeding a person who might not be able to digest solid foods. Partial hydrolysis of proteins either by using site-specific enzymes (such as trypsin or chymotrypsin) or by control of hydrolysis time, often improves foaming and emulsification properties, but not gelling properties. With some proteins, partial hydrolysis may cause a transient decrease in solubility, because of exposure of the buried hydrophobic regions.

The drawback of many protein hydrolysates, is that when hydrolysed, most of the food proteins liberate bitter tasting peptides, which affect their acceptability in certain applications. The bitterness is associated with their mean hydrophobicity. The intensity of bitterness depends on the amino acid composition and sequence and the type of protease used. Hydrolysates of hydrophilic proteins, such as gelatin; are less bitter than the hydrolysates of hydrophobic proteins, such as casein and soya proteins. Protease that show specificity for cleavage at hydrophobic residues, produce hydrolysates that are less bitter than those enzymes which have a broader specificity. Thus, thermolysin, which specifically attacks the amino side of hydrophobic residues, produces hydrolysates that are less bitter than those produced by low specificity trypsin, pepsin and chymotrypsin.

With this, we finish our study on proteins.

### **Check Your Progress Exercise 3**

1. Fill in the blanks:
  - a) Protein concentrate or isolate have an ----- concentration of proteins by selective removal of the non protein matter from the source.
  - b) The nutritional value of protein concentrates is expressed as -----  
-----.
  - c) One of the most important functional characteristics of SPC is -----  
-----.
  - d) The two nutritionally valued proteins that form a part of whey are -----  
----- and -----.

- e) The main drawback of hydrolysates is that it liberates bitter tasting -----  
-----.
2. What are the nutritionally attractive features of SPC?  
-----  
-----  
-----
3. Mention any two applications of:
- a) Whey Protein Concentrates  
-----  
-----  
-----
- b) Soy Protein Isolates  
-----  
-----  
-----
4. What characteristics make ISP a preferred soy product?  
-----  
-----  
-----
5. How is an isolate different from a concentrate?  
-----  
-----  
-----  
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#### **4.5 LET US SUM UP**

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Proteins, one of the major nutrients, are an essential component of our body tissues. Amino acids, the principal building blocks of protein, are twenty in number and vary in their composition, structure and properties. In this unit, we learnt about different types of proteins, i.e., sources of different types of proteins and functional properties. The important functional properties included viscosity and gelation. Viscosity refers to resistance to flow and is specifically crucial in preparation of fluid foods. Gelation refers to the process where denatured molecules aggregate to form an ordered protein network.

Finally, the unit focused on various protein concentrates, isolates, hydrolysates and their wide applications in food industry.

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## 4.6 GLOSSARY

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<b>Adsorption</b>	: A surface phenomenon that involves adhesion of the molecules of gases, dissolved substances, or liquids in more or less concentrated form to the surface of solids/liquids with which they are in contact.
<b>Amino acids</b>	: Monomeric units of proteins, having both amino and carboxylic groups in their structure.
<b>Gelation</b>	: Formation of an ordered protein network when denatured molecules aggregate.
<b>Hydrogen Bonding</b>	: Non-covalent interaction between two opposite charged groups. In proteins these are the major determinant of the secondary structure.
<b>Hydrolysate</b>	: Breakdown of proteins progressively to yield peptides or finally amino acids by the action of enzymes or chemical agents.
<b>Hydrophobic Interaction</b>	: Major determinant of tertiary structure of proteins
<b>Proteins</b>	: Complex nitrogenous compounds which consist of amino acids linked together by peptide bonds. They are the basic constituents of all living things and are a necessary part of the part of the food of all animals.
<b>Single cell Protein</b>	: Proteins from sources, like algae, fungi, bacteria and yeast
<b>Viscosity</b>	: Resistance to flow.

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## 4.7 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

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### Check Your Progress Exercise 1

1. Proteins can be classified based on:
  - Shape and size: fibrous or globular proteins.
  - Functional properties: Immuno, contractile, respiratory, structural, enzymatic, hormonal and carrier proteins.
  - Solubility and physical properties: Simple, conjugated and derived proteins.
  
2. The important functions of proteins in biological systems are as follows:  
(any two):
  - enzymes
  - membrane carriers
  - antibodies
  - structural element
  - hormonal messenger
  
3. Protein food sources can be divided into the following three categories:
  - Proteins of plant origin example meat, milk, eggs, fish etc.
  - Proteins of plant origin such as vegetable proteins, cereal proteins, seed proteins and nuts.
  - Single Cell Proteins.
  
4. The proteins obtained from microbial sources, i.e. algae, fungi, bacteria, yeast etc. are referred to as Single Cell Proteins. These are isolated from microorganisms. Some of the advantages of selecting microorganisms as a source of protein are:
  - High yield of proteins on dry weight basis.
  - Cost effective nutritional requirement.
  - Less area is required for the installation of plant for the production of proteins.
  - The plant may be designed in such a way that the processing can be done on a continuous basis instead of batch to batch basis.
  
- 5.

- a) Homoproteins; Heteroproteins
- b) alcohol dehydrogenase
- c) mechanochemical role
- d) keratins, collagens, elastins
- e) immunoglobulins
- d) Quaternary structure
- e) Sarcoplasmic, contractile, stroma; sarcoplasmic, myofibrillar, connective tissue.
- f) Structural component, food reserve; structure and protection to kernel.

1. Primary, Secondary, Tertiary and Quaternary are the different levels in which the protein structure is divided into.
2. The advantages of selecting micro-organisms as a source of protein are: high yield of proteins on dry weight basis; nutritional requirement is quite cheap; less area is required for the installation of plant for the production of proteins; and processing can be done on a continuous basis.
3. The major drawback of using algae as the source of protein is that it has a cellulosic cell wall, which is not digestible by human beings, thus causes nausea, vomiting, abdominal pain etc.
- 4.

### **Check Your Progress Exercise 2**

1.
  - a) facilitating fluidity, extensibility and expansion; elasticity, cohesiveness and mixing tolerance.
  - b) hydration, surface, protein-protein interactions
  - c) pH, ionic strength, solvent and temperature
  - d) foam foods
  - e) emulsification, whipping and film formation.

2. Those physico-chemical properties that enables the protein to contribute to the desirable properties/ characteristics of food are termed as functional properties. A few functional properties of proteins are:
  - Hydration properties that includes solubility, viscosity, gelation and texturization
  - Protein-protein interactions which include dough formation
  - surface properties which include emulsifying properties etc.
  
3.
  - a) Gelation refers to the process where the denatured molecules aggregate to form an ordered protein network by balancing protein-protein and protein-solvent interactions in the food products. These gel matrices can hold other food ingredients in processing food products, such as, dairy products, meat or fish products, vegetable proteins texturized by extrusion or spinning and bread dough.
  
  - b) Texturization process lead to film or fiber-like products with chewiness and good water holding capacity along with an ability to retain these during hydration and heat treatment. Texturized products are often used as meat substitutes and/or substitutes.
  
  - c) In foams, proteins form a protective layer (film) by adhering at the gas liquid interface and thus prevent the foam to collapse, thus act as a stabilizer.

### **Check Your Progress Exercise 3**

1.
  - a) increased
  - b) Protein Efficiency Ratio
  - c) Water-binding capacity, fat-binding capacity and emulsification properties
  - d)  $\alpha$ -lactalbumin and  $\beta$ -lactoglobulin
  - e) peptides

2. The nutritionally attractive features of SPC are high protein content, the near absence of anti-tryptic and anti-nutritional factors, absence of flatulence and substantial dietary fibre content.
  
3.
  - a) The applications of Whey Protein Concentrates are as a health supplement; bakery products; ice creams; processed cheese.
  - b) The applications of Soy Protein Isolates are in the following products meat; seafood; cereal; dairy-type products and infant formulas.
  
4. The characteristics which make ISP a preferred product are: blandness, absence of flatus-producing sugars and negligible fibre content.
  
5. An isolate contains not less than 90% protein on a moisture-free basis while concentrate contains not less than 70% protein on a moisture-free basis.