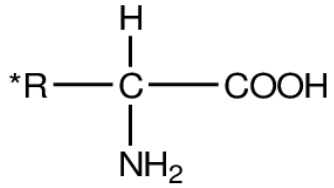
LECTURE 6: Proteins

• Macromolecular polymers composed of amino acids as the basic unit linked by peptide bonds
• Fundamental structural units of all proteins
• Contain carbon, hydrogen, oxygen, nitrogen, and sulfur, and some minerals
• May be present as simple or complexes with lipids or nucleic acids Importance of Proteins
• Found in all living cells and constituent of protoplasm, cell membrane, and nuclear material
• Constitute about 20% of the animal body
• Play an important role in many biochemical, biophysical, and physiological processes
• Enzymes are specialized proteins with catalytic activities present in all living cells
• Serve as regulators of metabolic reactions indirectly in the form of chemical messengers known as hormones
• Are transport agents
• Antibodies are proteins Amino Acids
• Building blocks of proteins are amino acids
• There are 20 amino acids that are protein constituents
• Contain a basic amino group and an acidic carboxyl group attached to the same carbon atom
• Possess a third group referred to as the side chain denoted by the letter R

Structural Formula of amino acid



Properties of Amino Acids

• White crystalline substances
• Aliphatic and aromatic compounds are readily soluble in polar organic solvents
• Have high melting points (200-300°C)
• Amino acids are amphoteric compounds
• Amino acids possessing both positive and negative charges are called Zwitterions
• The pH at which the amino acid has no tendency to move either towards positive or negative electrode is called isoelectric pH Properties of Amino Acids
• All amino acids possess asymmetric carbon atom except glycine
• The D and L forms refer to the stearic configuration of amino group
• All proteins are formed from 20 different amino acids, and they have common names based on the source from which they were first isolated
• The 20 amino acids found in proteins are referred to as standard or protein amino acids Non-polar (or) Aliphatic R groups • Includes glycine, alanine, valine, leucine, isoleucine, and proline

• Hydrocarbon R groups are non-polar
• These amino acids tend to fold inside away from the aqueous phase
• Promote hydrophobic reactions with protein structures
• Proline reduces the structural flexibility of the protein Non-polar Aromatic R groups
• Includes phenylalanine, tyrosine, tryptophan
• Participate in hydrophobic interactions that are stronger than aliphatic R groups
• Tyrosine (hydroxyl) and tryptophan (nitrogen in indole ring) are more polar
• Absorption of ultraviolet (UV) light at 280 nm is useful in the characterization and quantification of proteins Amino Acids Polar Uncharged R groups
• Includes serine, threonine, cysteine, methionine, asparagine, and glutamine
• Serine (Ser), threonine (OH), cysteine (Cys), methionine (Met) (sulfur), asparagine, glutamine (amide) contribute to the polarity
• The R groups are more hydrophilic than the non-polar amino acids Polar Uncharged Amino Acids.

Reactions involving proteins and alkali

5.2 Some common changes are described below

5.2.1 Denaturation

Denaturation is a phenomenon that involves transformation of a well-defined folded structure of protein to an unfolded state, without any change in the primary structure. Most food proteins are denatured when exposed to moderate heat treatments (60°C-90°C/1 h or less).

Denaturation is generally reversible when the peptide chain is stabilized in its unfolded state by the denaturing agents and the native conformation can be restabilized after the removal of the agent. Irreversible denaturation occurs when the unfolded peptide chain is stabilized by interactions with other chains.

The predenatured transition state involves minor conformational changes that occur prior to denaturation. As the reaction proceeds, changes due to denaturation occur. Following these changes, the protein may react either with themselves and/or with other food constituents resulting in the formation of higher molecular weight aggregates. These post-denaturation reactions are virtually irreversible.

Changes resulting from these mild heat treatments are usually beneficial from a nutritional standpoint, e.g.

- Digestibility is often improved. In general denatured proteins are more readily attacked by proteolytic enzymes.
- Several enzymes like proteases, lipoxygenases, polyphenol oxidases, etc. are inactivated. This limits the undesirables changes like development of off-flavours, acidity, textural changes and discoloration of foods during storage.
- Proteinaceous anti-nutritional factors present in seeds and legumes are denatured and inactivated by mild heat treatments. These inhibitors impair efficient digestion of proteins and thus reduce their bioavailability.
- Certain proteinaceous toxins, e.g. botulism toxin and enterotoxins are inactivated.

However, extensive denaturation affects certain functional properties like solubility and other related properties.

5.2.2 Desulfuration

Thermal treatments of proteins or proteinaceous foods at high temperature and in the absence of any added substances can lead to several chemical changes. Most of these chemical changes are irreversible and some of these reactions result in the formation of amino acid types that are potentially toxic. One of the first noticeable changes in proteins on heating at around 100°C is loss of heat-labile amino acids such as cysteine, cystine & lysine and the formation of gases like hydrogen disulphide (H_2S). Thermal treatments like sterilization at temperature above 115°C bring about the partial destruction of cysteine and cystine residues and formation of H_2S, dimethyl sulfide and cysteic acid; H_2S and other volatile compounds produced contribute to the flavor of these heat treated foods.

5.2.3 Deamidation

This reaction takes place during heating of proteins at temperatures above 100°C. The ammonia released comes mainly from the amide groups of glutamine and asparagine, and these reactions do not impair the nutritive value of the proteins. However, due to the unmasking of the carboxyl groups, the isoelectric points get affected and therefore the functional properties of proteins are modified. Deamidation may be followed by establishment of new covalent bonds between amino-acid residues.

5.2.4 Racemization

Severe heat treatment at temperatures above 200°C as well as heat treatment at alkaline pH (e.g. in texturized foods) invariably leads to partial racemization of L-amino acid residues to D-amino acid residues. Some racemization is also observed during acid hydrolysis of proteins and roasting of proteins or protein containing foods above 200°C. Since D-amino acids have no nutritional value, racemization of an essential amino acid reduces its nutritional value by 50%. Racemization of amino acid residues causes a reduction in digestibility because peptide bonds involving D-amino acid residues are less efficiently hydrolyzed by gastric and pancreatic proteases. This leads to loss of essential amino acids that have racemized and impairs the nutritional value of the protein. D-amino acids are also less efficiently absorbed through intestinal mucosal cells and even if absorbed they can't be utilized in in-vivo protein synthesis.

5.2.5 Effect of heat treatment at alkaline pH

Alkali treatment causes many reactions (undesirable reactions). The more common ones are hydrolysis, elimination reactions involving side chains of certain amino acids, racemization of amino acid residues, addition of compound to the proteins, scission of the peptide chain, modification or elimination of non protein constituents (prosthetic groups etc.), and the interaction of the protein with alkali-derived products from the environment. All of these reactions are affected by the pH, the temperature, ionic strength, presence of specific ions, and by the nature of the protein itself. . Heating of proteins at alkaline pH or heating above 200°C at neutral pH can result in β-elimination reaction. The first stage of this reaction

involves abstraction of proton from α-carbon atom resulting in formation of carbanion. The carbanion derivative of cysteine, cystine and phosphoserine undergoes second stage of β-elimination reaction leading to formation of dehydroalanine.

ENZYME CATALYZED REACTIONS INVOLVING HYDROLYSIS AND PROTEOLYSIS

6.1 Introduction

Processes involving proteolysis play an important role in the production of many foods. Proteolysis can occur as a result of proteolytic enzymes present in the food itself or those from microbial sources. This large group of enzymes is divided into two large subgroups

1. **Peptidases (exopeptidases)** - These enzymes cleave amino acids or dipeptide in a stepwise manner from the terminal end of protein.
2. **Proteinases (endopeptidases)**- These enzymes hydrolyze the linkages within the peptide chain and do not attack terminal peptide bonds.

6.2 Types of proteolytic enzymes

Proteolytic enzymes can be divided into four groups: the acid proteases, the serine proteases, the sulfhydryl proteases, and the metal containing proteases.

6.2.1 Acid proteases

Those that have pH optimum at low pH. e.g. pepsin, rennin (chymosin). In the dairy industry, in cheese manufacture, thef ormation of casein curd is achieved with chymosin or rennin. Rennin is present in the fourth stomach of the suckling calf. Rennin can also be produced by genetically engineered microorganism. The coagulation of milk by rennin occurs in two stages. In the first, enzymatic stage, the enzyme acts on κ-casein (hydrolysis of peptide bond between Phe_{105}-Met_{106}) resulting in the formation of insoluble para-κ-casein and a soluble glycomacropeptide. The second stage involves the clotting of the modified casein micelles by calcium ions. Rennin is essentially free of other undesirable proteinases and is, therefore, especially suitable for cheesemaking.

6.2.2 Serine proteases

They have the presence of a serine and a histidine residue in their active sites. e.g. chymotrypsin, trypsin, plasmin, thrombin. Serine proteases are produced by a great number of bacteria and fungi. Chymotrypsin and trypsin are pancreatic enzymes that carry out their function in the intestinal tract. Trypsin cleaves linkages of amino acid residues with a basic side chain (lysyl or arginyl bonds).

6.2.3 Sulfhydryl proteases

Require sulfhydryl group (–SH) for activity. They are mostly of plant origin e.g. papain, ficin, bromelain. The active sites of these plant enzymes contain a cysteine and a histidine group that are essential for enzyme activity. These enzymes catalyze the hydrolysis of peptide, ester and amide bonds. Haze is a result of the combination of polypeptide and tannin molecules in beer giving rise to easily observed particles. Proteolytic enzymes (papain, ficin, bromelain) prevent this type of haze by reducing the polypeptide size.

6.2.4 Metal containing proteases

These enzymes are exopeptidases. They require a metal for activity and are inhibited by metal chelating compounds e.g. aminopeptidases, carboxypeptidases A and B, dipeptidases. Most of these enzymes contain zinc. Carboxypeptidases remove amino acids from the end of peptide chains that carry a free α-carboxyl group. Aminopeptidases remove amino acids from the free α-amino end of the peptide chain.

6.3 Application of proteolytic enzymes in foods

Enzymes are used for protein hydrolysis to:

1. To provide a wide variety of proteins known as enzymatically modified proteins e.g. egg protein, whey protein
2. Improving functional properties of proteins
3. For solubilization of denatured proteins
4. For maintenance of protein solubility in acid media
5. Increasing digestibility
6. Decomposition of those proteins that possess undesirable properties.