FOOD PROTEINS

Classification and physicochemical properties

Introduction

Proteins are common constituent of all biological materials, without which life is not possible. They are essential constituent of all living cells. A complex nitrogenous organic compound − a polymer of amino acids – therefore defined as high molecular weight polymers of low molecular weight monomers known as amino acids, which are linked to gether by peptide bonds. Proteins are polymers of some 20 different amino acids joined together by peptide bonds (primary structure). The amino acid composition establishes the nature of secondary and tertiary structures. These, in turn, significantly influence the functional properties of food proteins and their behaviour during processing.

Classification of Proteins

Proteins have been classified in many ways. Generally they are classified on the basis of composition, shape of molecules and solubility.

On the basis of composition

On the basis of composition proteins are classified into three groups *viz.* simple proteins, conjugated proteins and derived proteins.

1. Simple proteins

These are the proteins which consist of only amino acids − They do not contain other class of compounds.

2. Conjugated proteins

These are the proteins which consist of amino acids as well as other class of compounds. They are further classified into six subgroups.

Conjugated proteins

Sr. No.	Class	Other compound present	Example
1	Chromoprotein	Coloured pigment	Haemoglobin
2	Glycoprotein	Carbohydrate	Mucin (in saliva)
3	Phosphoprotein	Phosphoric acid	Casein (in milk)
4	Lipoprotein	Lipid	Lipovitelin (in egg yolk)
5	Nucleoprotein	Nucleic acid	Viruses
6	Metalloprotein	Metal	Ciruloplasmin (Cu)

Derived proteins

They represent various stages of hydrolytic cleavage of simple or conjugated proteins. e.g. proteoses, peptones, peptides, etc.

On the basis of shape of molecules

On the basis of shape of molecules, proteins are classified into two main groups viz. fibrous proteins and globular proteins.

1. Fibrous proteins

Fibrous proteins are long and thread or ribbon like and tend to lie side by side toform fibers. They are generally insoluble in water as the intermolecular forces in these proteins are rather strong. They serve as the chief structural material of animal tissues. Examples are keratin, myosin, collagen etc.

2. Globular proteins

Globular proteins are spheroidal in shape. They are generally soluble in water or aqueous solution of acids, bases or salts as intermolecular forces in these proteins are relatively weaker. These proteins are generally involved in physiological processes of the animal body. Examples are enzymes, some hormones, haemoglobin, etc.

On the basis of solubility

On the basis of solubility proteins are classified into the following groups.

1. Albumins-These proteins are soluble in distilled water, dilute salt, acid and base solutions. Examples are lactalbumin, egg albumin.

2. Globulins- These proteins are insoluble in distilled water, but soluble in dilute salt, acid and base solutions. Examples are serum globulins and β-lactoglobulin in milk, myosin and actin in meat.

3. Protamine and Histones-These proteins are highly soluble in distilled water. These are small molecules, stable to heat (i.e. not coagulated by heat). Protamine soluble in NH_4OH, whereas histones insoluble NH_4OH.

4. Glutelins - These proteins are insoluble in distilled water and alcohol but soluble in dilute acid and base solution. Examples are glutenin in wheat, oryzenin in rice.

5. Prolamins - These proteins are insoluble in distilled water, but soluble in dilute acid, dilute base and 70-80% alcohol. Example are zein in corn, gliadin in wheat.

6. Scleroproteins - These proteins are insoluble in most of the solvents like water, dilute acid, dilute base, dilute salt solution etc. They are generally fibrous proteins serving structural and binding purposes. Examples are collagen, elastin, keratin.

Physicochemical properties of proteins

1. Isoelectric point:

The isoelectric point of a protein is that pH at which the net charge on the protein molecule is zero. At isoelectric point protein will not migrate when an electric field is applied. At isoelectric point its ionization is minimum − least soluble. Each protein have its own characteristic isoelectric point − due to difference in amino acids make up. The major milk protein casein has an isoelectric point of 4.6. This character of protein is often made use in the isolation of proteins.

2. Amphoteric behaviour

Like amino acids, proteins are ampholytes, i.e. they act as both acids and bases. At all but the extremes of pH, possess both positive and negative charged groups. Owing to the presence of carboxylate groups of the acidic amino acids ---- carboxylate group at the end of the chain, most protein solutions are good buffers below pH 5. Similarly owing to the ε-amino groups of lysine, the guanidinium group of arginine and the phenolic hydroxyl group of tyrosine, most proteins are good buffer at pH values above 9. However at neutral pH values, most proteins have limited buffering capacity. This buffering is of great importance in many living tissues.

3. Ion binding

As ampholytes, proteins can bind both anions and cations. Several ions will form insoluble salts with proteins and this phenomenon is widely used to remove proteins from solutions. e.g. Trichloro acetic acid is used to separate protein nitrogen from non protein nitrogen. It is possible to obtain interactions between proteins and charged macromolecules such as alginates and pectates. These type of complexes have great potential in the food.

4. Solubility

As would be expected for an ampholyte, protein solubility is markedly dependent on the pH and ionic composition of the solution. Protein solubility is minimal at the isoelectric point since at this pH the net charge on the protein is zero and consequently electrostatic repulsive forces are minimal while

interaction between protein molecules is maximal. Relationship between salt concentration and solubility is complex. Globulins which are soluble in 5-10 % salt solutions, are insoluble in water while albumins are readily soluble in both water and dilute salt solutions. However, in concentrated salt solution ; all proteins become less soluble.

The increase in solubility in dilute salt solution observed with globulins is known as "salting – in". It can be explained in terms of the relative affinity of the protein molecules for each other and for the solvent. i.e. the ions of the neutral salt will interact with the protein; thereby decreasing protein-protein interactions and consequently increasing the solubility.

The decreasing solubility of proteins at high salt concentration is known as "salting out". Dehydration of the protein molecules occur due to the added salt. The large number of salt ions in the solution will 'hydrate' and organise water molecules around them, thus reducing the water available for the protein molecules. Since protein solubility depends on whether 'clustering' around the hydrophilic groups, the 'dehydrated' proteins will precipitate. In an aqueous protein solution not all the water will be 'free' as some will be 'bound' to the protein via hydration of charged groups and hydrogen bonds.

5. Swelling

Several native proteins which are not soluble in water may, however, interact with aqueous solution to form swollen, gel like systems, examples being actomyosin and collagen in muscles. There are two mechanisms whereby this swelling occurs.

(i) Osmotic (Donnan swelling) − which is reversible and caused by interactions between ions and charged sites on the protein. To maintain electrical neutrality in the swollen phase, small ions of opposite charge migrate from the solution to the swollen phase. These excess ions in the swollen phase give rise to an osmotic pressure which causes the swelling.

(ii) Lyotropic swelling − which is irreversible and caused by non ionic reagents which act by altering the water structure around the protein, interrupting the hydrogen bonds and / or through direct competition with internal hydrophobic interactions.

The swelling of insoluble proteins by these mechanisms will continue until it is restrained by the intermolecular forces between the protein molecules and an equilibrium swollen volume is achieved. Thus, both soluble and insoluble proteins can immobilise water and this ability to bind water is often called their water holding or water binding capacity.

6. Crystallization

Many of the proteins have been obtained in crystalline condition. Amongst the animal proteins haemoglobin crystallise readily. Many of the enzyme proteins have been crystallized e.g. urease, pepsin, trypsin, catalase etc. The crystallization of protein may be obtained by addition of a salt such as ammonium sulphate or sodium chloride and adjustment towards isoelectric pH. The addition of definite amount alcohol or acetone is occasionally advantageous. The added substances and adjustment to isoelectric pH decrease the solubility of the protein. The protein is also least dissociated at the isoelectric pH and crystallize best in the form of protein salts. The relative ease of crystallization of protein as compared to polysaccharides is due to the high polarity of the protein molecules giving rise to strong field of force which orient the molecules and promote crystal formation.

7.Optical activity

All the amino acids occurring in nature except glycine, contain one or more asymmetric carbon atom and therefore show optical activity. The rotatory power of amino acid is affected by various factors which influence the degree and the nature of the electrolytic dissociation of the amino acid. These include

- The concentration of amino acid itself.
- pH of solution.
- The nature of solvent.
- The presence of electrolytes.
- The temperature.

The effect of varying conditions is so large that any statement regarding the specific rotation of an amino acid has little meaning, unless accompanied by the statement of the conditions prevailing in the solution. Optical rotation is an important property of proteins in which they differ widely. This

phenomenon results from the presence of asymmetric carbon atom. Specific rotations of proteins obtained at 20 ^{o}C and using Dline of sodium are always negative and for globular proteins the values of $[\alpha]_{D\ 20}$ are usually within the range of -30^{o} to -60^{o}. Denaturation of proteins produces marked increases in optical rotation. Measurement of this property is a sensitive means of following denaturation.

8. Absorption of ultra violet light

The absorption of ultra violet light with a wavelength of 280 nm is a characteristic of proteins that depends on their content of the aromatic amino acids (tyrosine, tryptophan and phenylalanine).

9. Refractive index

The refractive index of protein solutions increases linearly with concentration. The difference between the refractive index of a 1 % protein solution and its solvent is called specific refractive increment. Most proteins have a refractive index increment of about 0.0018.

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Reactions involved in processing and reactions with alkali

Introduction

A number of chemical changes involving proteins may occur during processing and storage of foods. These changes can be desirable or undesirable. The various treatments involved in processing of foods are heating, cooling, drying, fermentation, use of chemicals, irradiation, etc. Among these, heating is most common processing treatment. Heating is mainly done to kill pathogens, inactivate enzymes that cause oxidative and hydrolytic changes in foods during storage. As a result of these chemical changes, nutritive value of proteins may be decreased.

- Formation of toxic compounds
- Destruction/ loss of amino acids
- Conversion of essential amino acids into derivatives which are not metabolizable
- Decrease in digestibility of proteins due to cross linking

The nature and extent of chemical changes induced in proteins by food processing depends on a number of parameters like composition of food and processing conditions like temperature, pH or presence of oxygen. As a consequence of these reactions, the biological value of proteins may be decreased.

Some common changes are described below

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_o-90_oC/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.

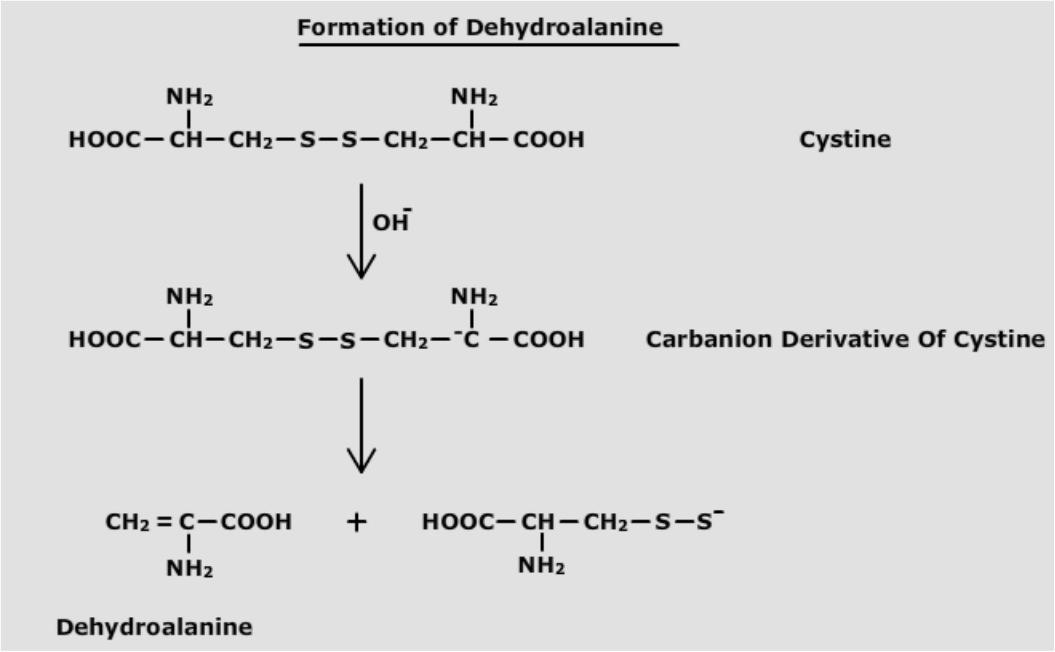
2. Desulfuration: Thermal treatments of proteins or proteinaceous foods at high temperature and in the absence of any added substances can lead to several chemical changes. Most of these chemical changes are irreversible and some of these reactions result in the formation of amino acid types that are potentially toxic. One of the first noticeable changes in proteins on heating at around 100_oC 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_oC 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.

3. Deamidation: This reaction takes place during heating of proteins at temperatures above 100_oC. 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.

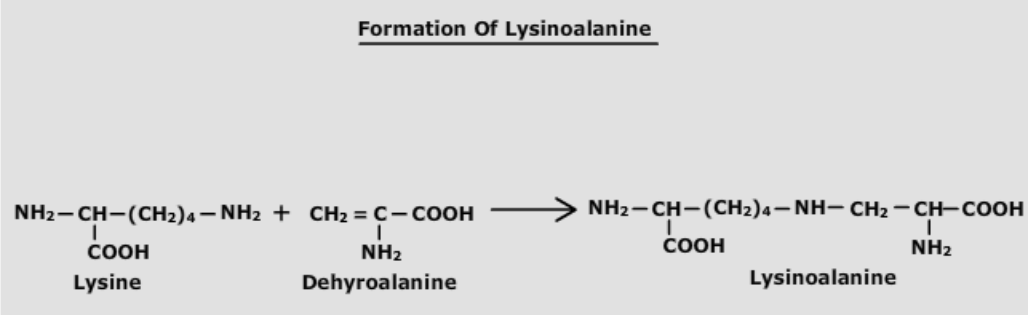
4. Racemization: Severe heat treatment at temperatures above 200_oC as well as heat treatment at alkaline pH (e.g. in texturized foods) invariably leads to partial racemization of L-amino acid residues to D-amino acid residues. Some racemization is also observed during acid hydrolysis of proteins and roasting of proteins or protein containing foods above 200_oC. 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 invivo protein synthesis.

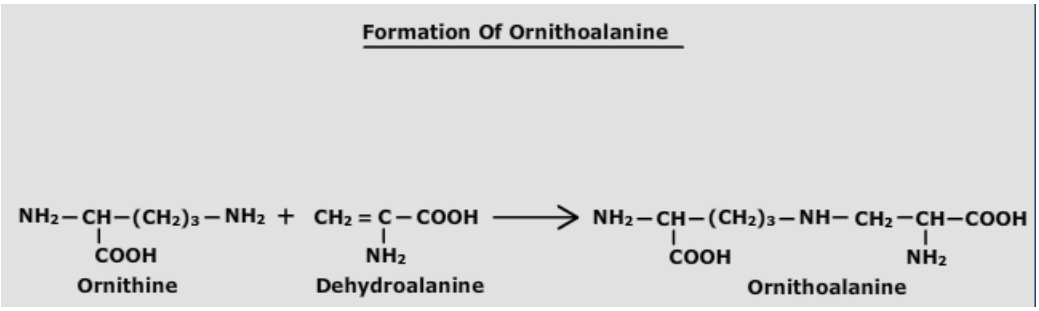
5. Effect of heat treatment at alkaline pH: Alkali treatment causes many reactions (undesirable reactions).The more common ones are hydrolysis, elimination reactions involving side chains of certain amino acids, racemization of amino acid residues, addition of compound to the proteins, scission of the 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 t ese 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$_o$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. The resulting dehydroalanine residues are very reactive and react with nucleophilic groups such as ε-amino group of lysine, thiol group of cystein and delta-amino group of ornithine (degradation product of arginine).These reactions results in formation of lysinoalanine, lanthionine and ornithoalanine cross-links respectively in proteins. Of these lysino-alanine is the major cross-link commonly found in alkali treated proteins because of the abundance of readily accessible lysyl residues. Formation of protein-protein cross-links in alkali treated proteins decreases their digestibility and biological value. Decrease in digestibility is related to the inability of trypsin to cleave the peptide bond in lysinoalanine. Cross-links also impose steric constraints that prevent the hydrolysis of other peptide bonds in the neighbourhood of such cross links.



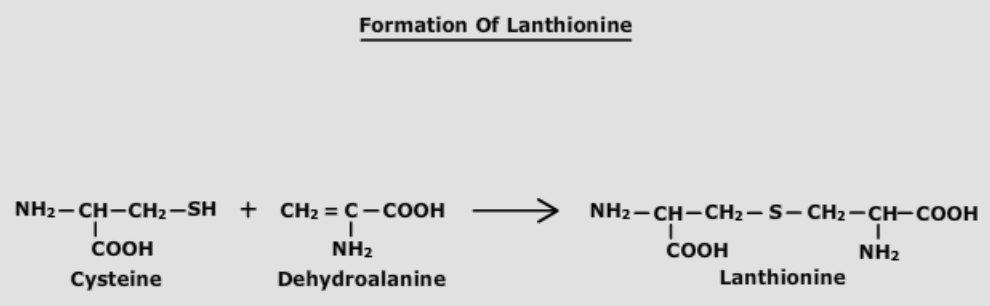
Formation of Dehydroalanine



Formation of Lysinoalanine



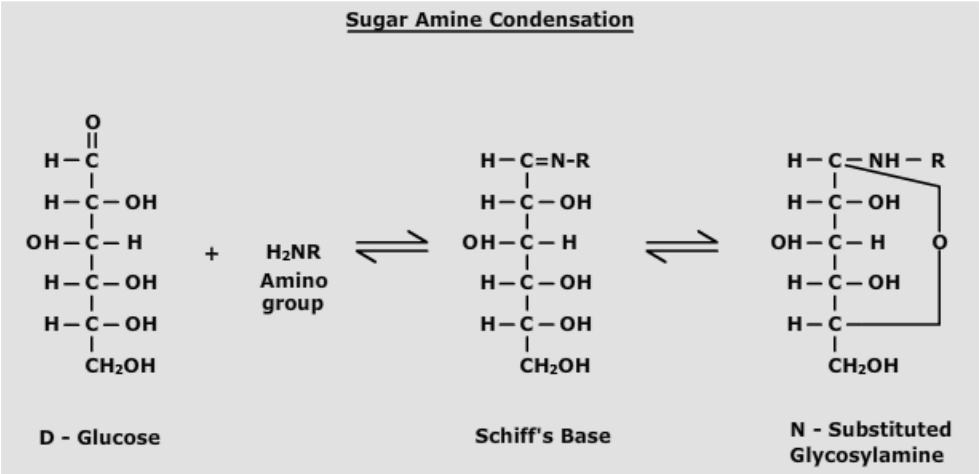
Formation of Ornithoalanine



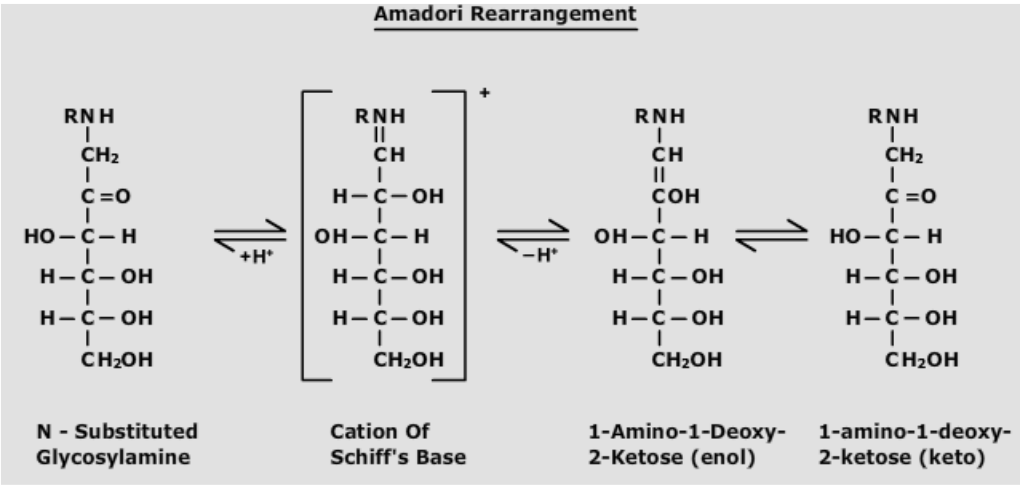
Formation of Lanthionine

6. Interaction between proteins and carbohydrates/aldehydes (Maillard reaction)

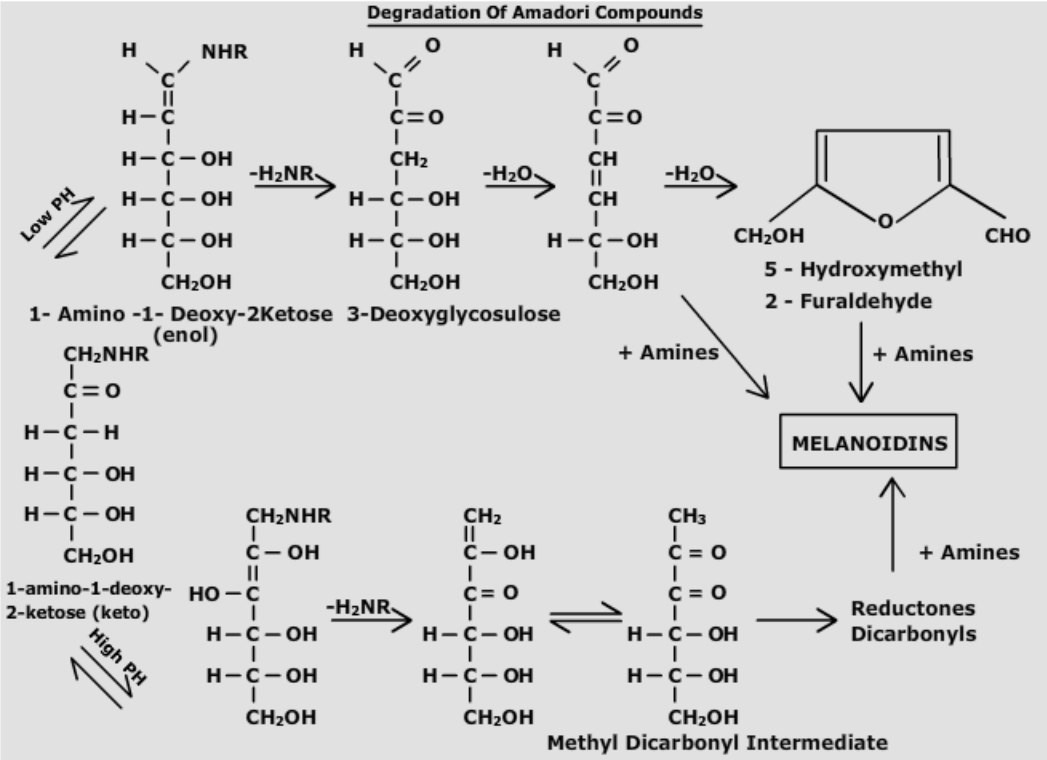
Maillard reaction (nonenzymic browning) refers to a complex set of reactions initiated by reaction between amines and carbonyl compounds, which, at elevated temperatures, decompose and eventually condense into insoluble brown products known as melanoidins. This reaction occurs not only in foods during processing but can also occur in biological systems. In either case, proteins and amino acids generally provide an amino component while reducing sugars, ascorbic acid and carbonyl compounds generated from lipid oxidation provide the carbonyl component.



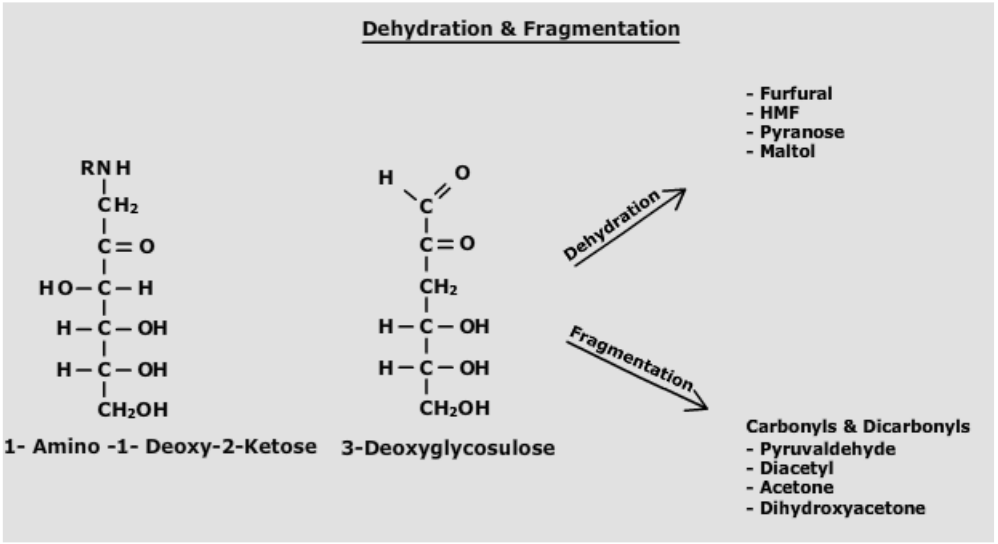
Sugar Amine Condensation



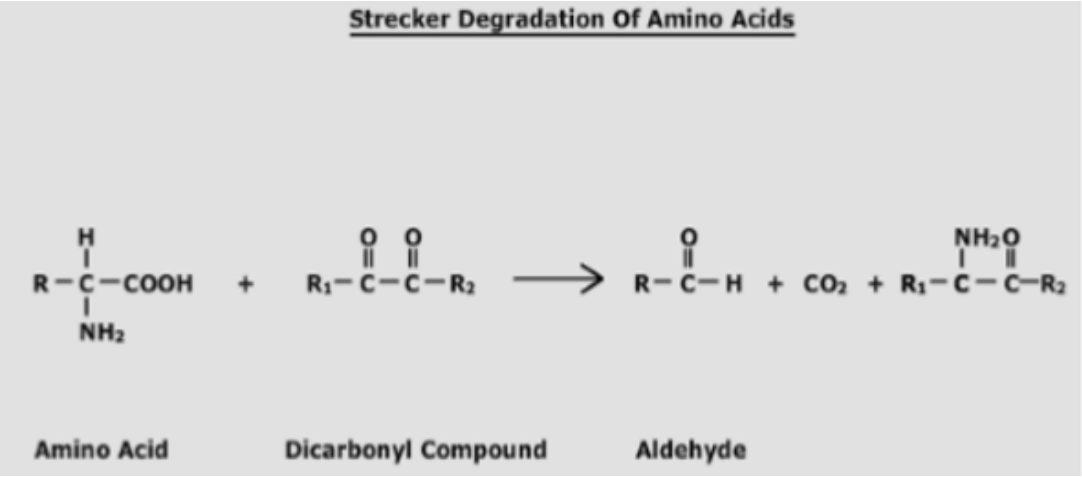
Amadori Rearrangement



Degradation of Amadori Compounds



Dehydration and Fragmentation



Strecker Degradation of Amino Acids

7. Significance of the Maillard Reaction

Maillard [Sugar − amino] type browning is most prevalent − because it requires relatively low energy of activation and is autocatalytic. Direct caramelization requires high energy of activation. Therefore occurs to a limited extent in food. Significance of Maillard reaction in food processing is given below.

1. **Production of colour**

Desirable as in coffee, chocolate bread crust, toast etc.

Undesirable, as in milk & milk products (khoa, condensed milk, milk powder etc) and in many intermediate moisture products.

2. Production of flavour and off flavour

Flavour (odour) are due to formation of volatile products e.g. fission products and strecker aldehydes. Substances tasting sweet & bitter may be involved.

3. Antioxidant properties

(i) Maillard reaction products are reported by have antioxidant properties.

(ii) This is thought to be due to formation of reductones, chelating of heavy metals, which may otherwise act as a proxidant.

4. Toxicity

(i) Through possible formation of imidazoles N-nitroso derivatives.

(ii) Some of the compounds are known to be carcinogenic in laboratory animals. Intrinsic toxicity is due to nutritional properties of Maillard products and intermediates.

5. Nutritional implications

One of the important reasons for interest of food industry in Maillard browning is its relation to nutrition.

Considerations in this regards are reduction in nutritive value.

Loss of essential amino acids - especially lysine.

Loss of some vitamins.

Increase excretion of Zn in urine due formation of metal chelating compounds.

Reduced digestibility due to development of cross-links between lactose and protein.

Inhibition of trypsin, carboxypeptidases (A and B) and amino peptidase by Maillard reaction production − metabolic inhibitors.

Inhibition of intestinal amino acid transport − disturbed amino acid utilization.

Lowered consumption of food due to prior palatability appearance and physical properties of the brown products.

8. Oxidation of amino acids Methionine is oxidized to methionine sulfoxide by various peroxides. Under strong oxidizing conditions, methionine sulfoxide is further oxidized to methionine sulfone, and in some cases to homocysteic acid.
