FOOD CHEMISTRY

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FOOD Chemistry

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Food Proteins

Proteins

Classification
and
physicochemical properties

- 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 together 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**(3), **shape of molecules**(2) and **solubility**(6).

On the basis of composition :

- proteins are classified into ***three grps,*** viz.simple proteins,conjugated proteins and derivedproteins.
- **1. Simple proteins** consist of only amino acids – They do not contain other class of compounds.
- **2. Conjugated proteins** consist of amino acids as well as other class of compounds and further classified into **six** subgroups.
- **3.Derived proteins** products of **hydrolytic cleavage** of simple or conjugated proteins. e.g. proteoses,peptones, peptides, etc.

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)

2.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 to form 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.

3.On the basis of solubility :

Proteins are classified into **six 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** in 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(asprtate, glutamate)** ---- 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 [α]D20] are usually within the range of -30o to -60o. **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**.

Reactions involved in processing and reactions with alkali

A number of **chemical changes** involving proteins may occur **during processing and storage** of foods.

❖These changes can be **desirable** or **undesirable**.

❖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 mainly done to **kill pathogens and inactivate enzymes** that cause oxidative and hydrolytic changes in foods during storage.

❖**As a result of these chemical changes** :

•Formation of **toxic** compounds (acrylamide from Asparagine + dicarbonyl)

• **Destruction/ loss** of amino acids

• **Conversion** of essential amino acids into derivatives which are **not metabolizable** (**L -- D**)

• Decrease in **digestibility** of proteins due to cross linking

• **nutritive value** of proteins may be decreased

❖ 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:** involves transformation of a well-defined **folded structure** of protein to an **unfolded** state , without any change in the primary structure , when exposed to moderate heat treatments (**60o-90oC/1 h or less**).

- Generally **reversible** 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 ---- 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**.

- 1. Changes resulting from these mild heat treatments are usually **beneficial** from a nutritional standpoint,

e.g. **Digestibility** is often **improved** (more readily attacked by proteolytic enzymes).

- 2. Several **enzymes** like proteases, lipoxygenases, polyphenol oxidases, etc. are **inactivated** which **limits the undesirable changes** like development of off-flavours, acidity, textural changes and discoloration of foods during storage.
- 3. Proteinaceous **anti-nutritional factors (phytate ,saponin,tannin)** 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.
- 4. Certain **proteinaceous toxins**, e.g. botulism toxin and enterotoxins are **inactivated**.
- 5. Extensive denaturation affects certain **functional properties** like solubility and other related properties.

2. Desulfuration: Thermal treatments can lead to several chemical changes **--- irreversible .**

- Heating at around **100oC --- loss** of heat-labile amino acids such as **cysteine, cystine & lysine** and the formation of gases like hydrogen disulphide (**H2S**).
- Sterilization at temp **above 115oC** bring about the **partial destruction** of cysteine and cystine residues and formation of **H2S**, **dimethyl sulfide** and **cysteic acid**; H2S and other volatile compounds produced contribute to the **flavor** of these foods.

3.Deamidation: Above **100oC** , **ammonia** released comes mainly from the **amide groups** of **glutamine and asparagine**, and these reactions **do not impair the nutritive value**.

- Due to the unmasking of the carboxyl groups, the **isoelectric points get affected** and therefore the **functional properties** of proteins are **modified**.

4.Racemization: Above 200oC, heat treatment at **alkaline pH, acid hydrolysis** and **roasting** invariably leads to **partial racemization of L-amino acid residues to D-amino acid residues**.

- 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:

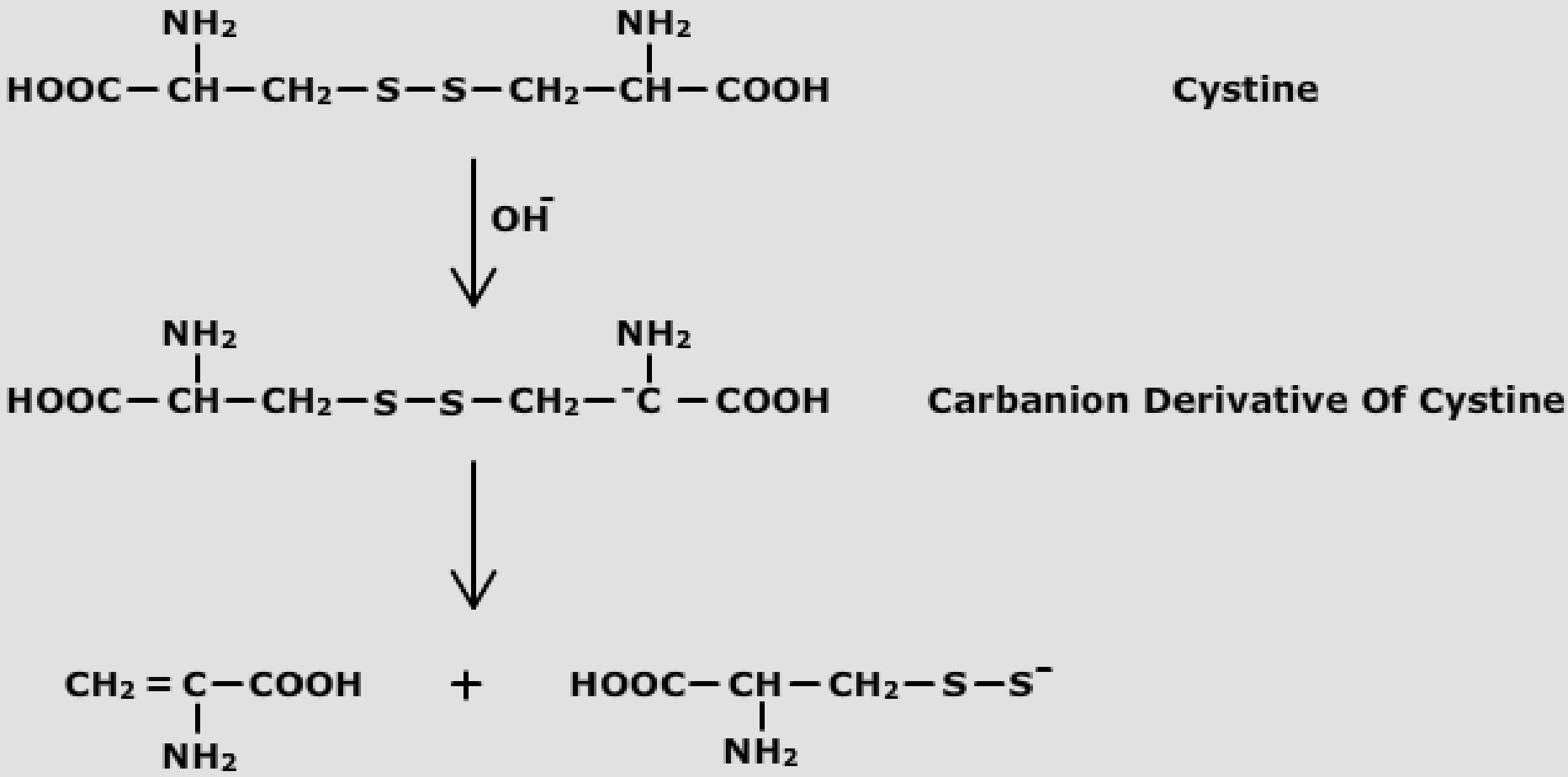
Undesirable reactions , like **hydrolysis** , **elimination reactions** involving side chains of certain amino acids, **racemization** of amino acid residues, **addition of compound** to the proteins, **scission of the peptide chain**, **modification** or **elimination** of non protein constituents and 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.

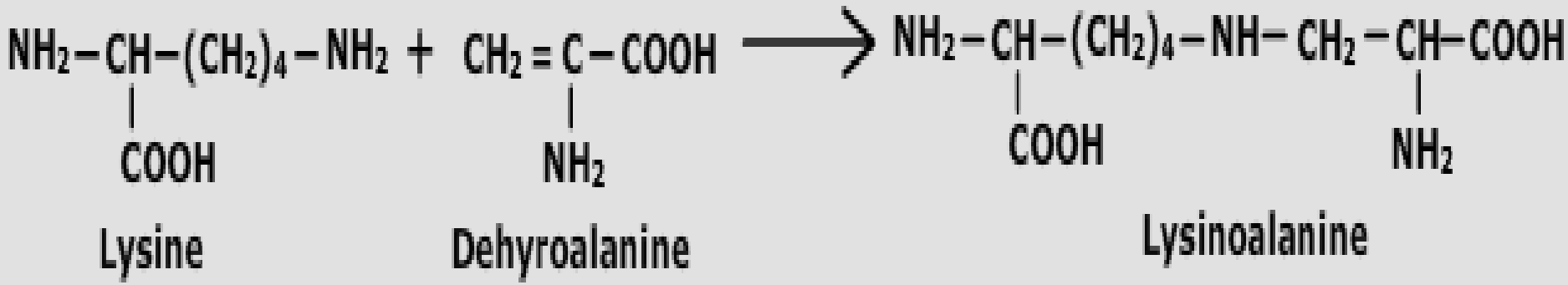
Heating of proteins at **alkaline pH** or heating **above 200oC** at neutral pH can result in **β-elimination reaction**. The **first stage** involves abstraction of proton from **α-carbon** atom resulting in formation of **carbanion**. The carbanion derivative of cysteine, cystine and phosphoserine **undergoes second stage** of **β-elimination reaction** leading to formation of **dehydroalanine** which react with **nucleophilic groups** such as amino group of **lysine,** thiol group of **cystein** and amino group of **ornithine** (degradation product of arginine) resulting 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 prevent the hydrolysis of other peptide bonds in the neighborhood of such cross links.

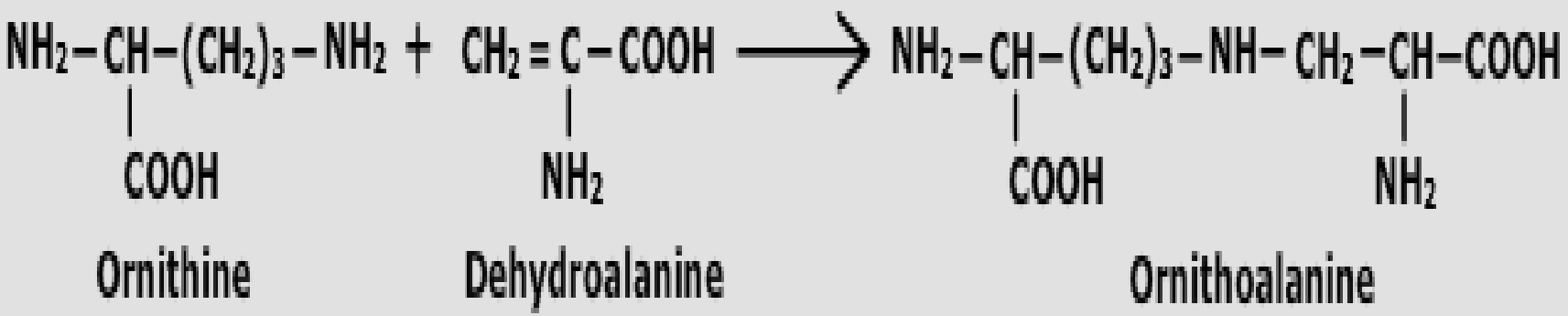
Formation of Dehydroalanine



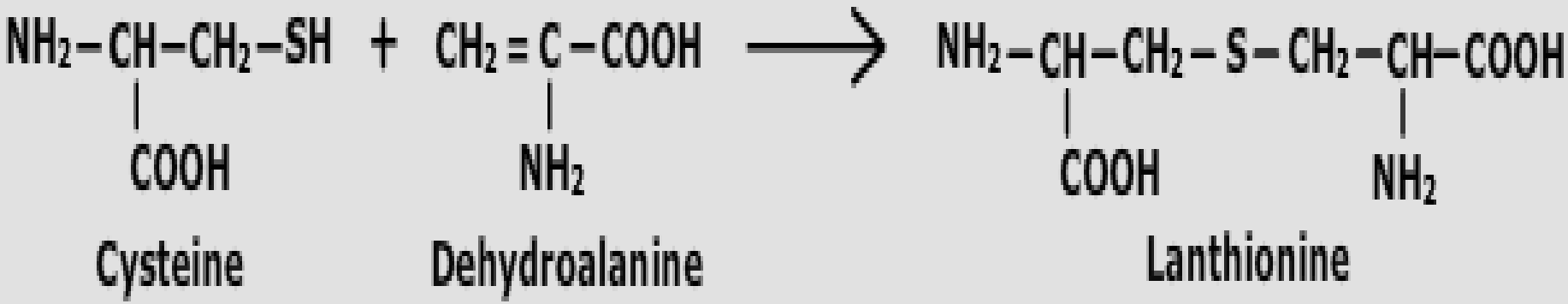
Formation Of Lysinoalanine



Formation Of Ornithoalanine



Formation Of Lanthionine



Interaction between proteins and carbohydrates/aldehydes (Maillard reaction) :

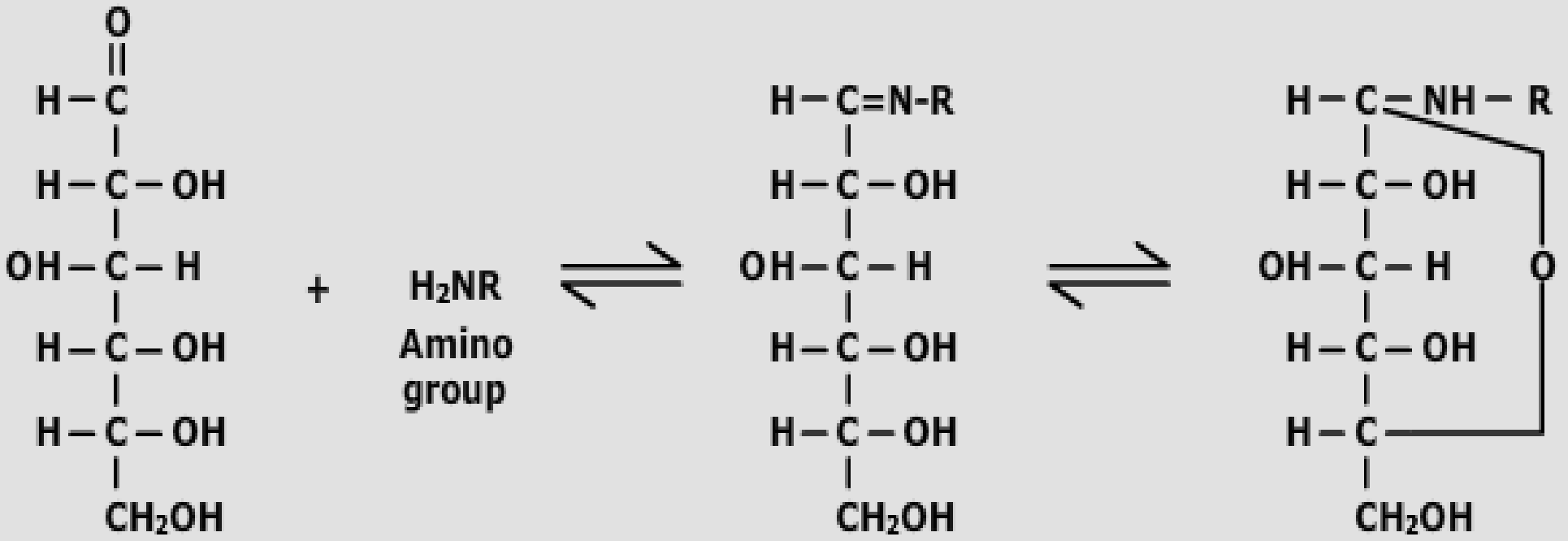
- Maillard reaction (nonenzymic browning) refers to a complex set of reactions.

- It is initiated by reaction between **amines** and **carbonyl compounds**, which, at elevated temperatures, **decompose** and eventually **condense** into **insoluble brown products** known as **melanoidins**.

- Proteins and amino acids generally provide an **amino component.**

- Reducing sugars, ascorbic acid and carbonyl compounds generated from lipid oxidation provide the **carbonyl component.**

Sugar Amine Condensation

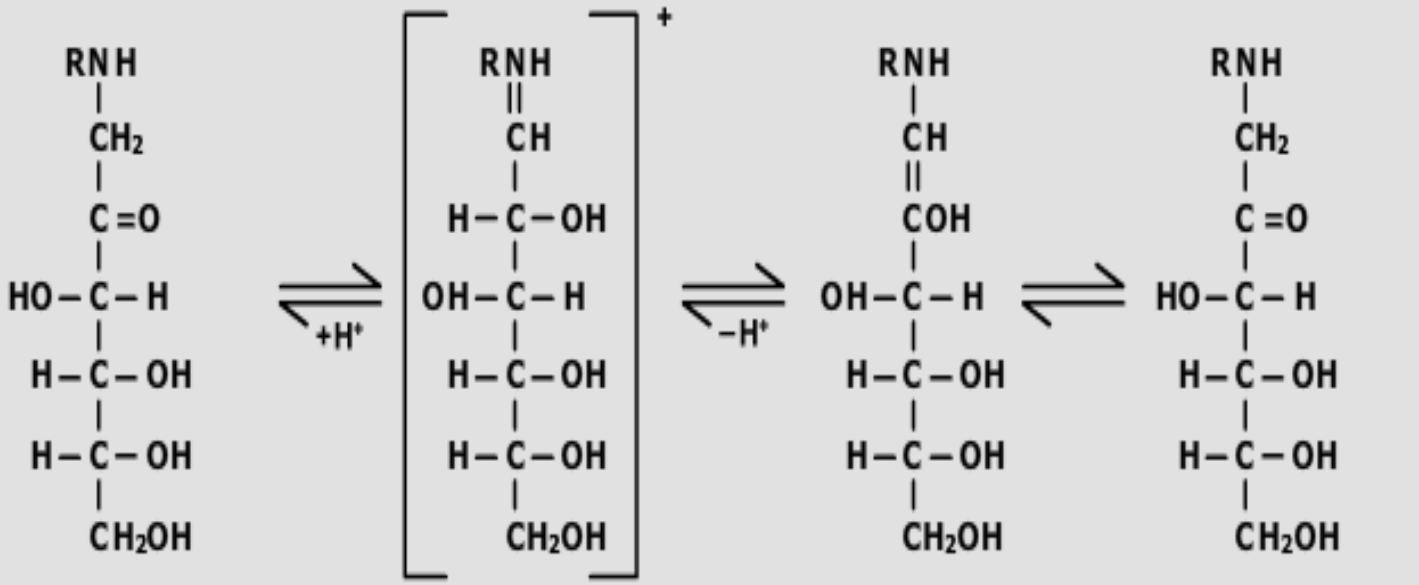


D - Glucose

Schiff's Base

N - Substituted Glycosylamine

Amadori Rearrangement

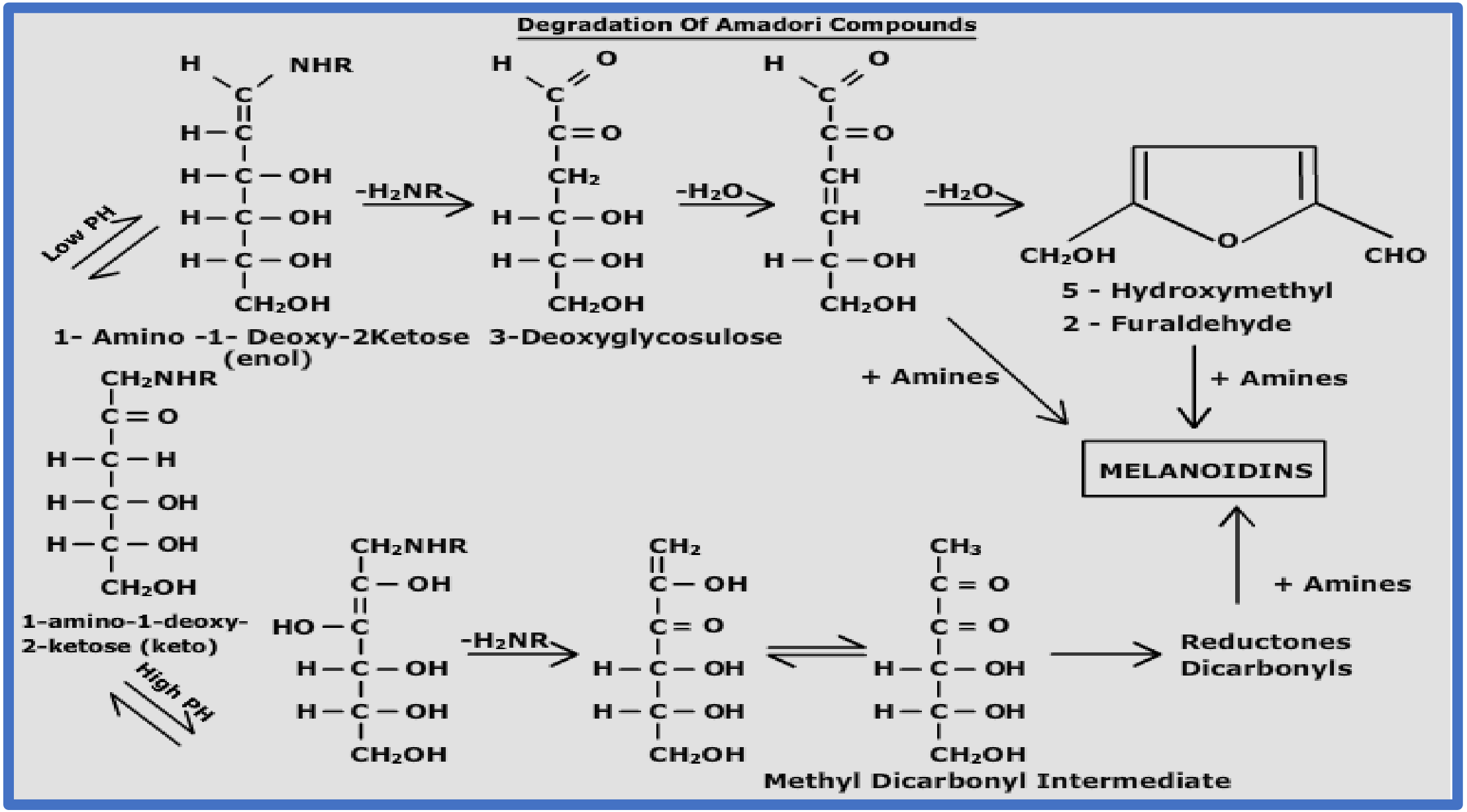


N - Substituted Glycosylamine

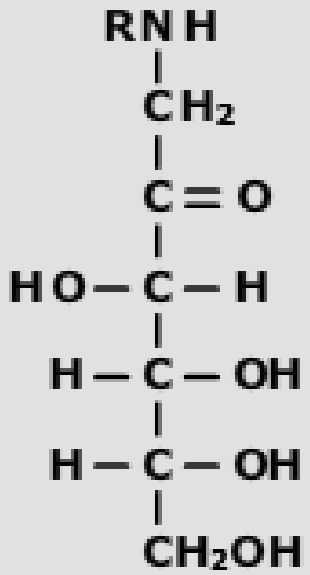
Cation Of Schiff's Base

1-Amino-1-Deoxy-2-Ketose (enol)

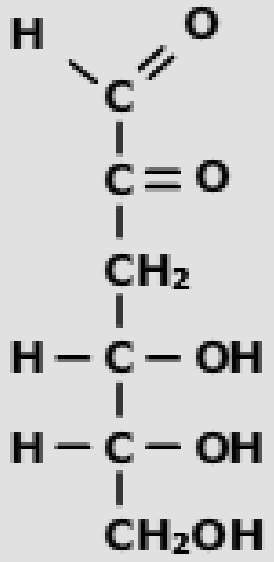
1-amino-1-deoxy-2-ketose (keto)


Degradation Of Amadori Compounds
Low PH
-H2NR
-H2O
-H2O
1- Amino -1- Deoxy-2Ketose (enol)
3-Deoxyglycosulose
5 - Hydroxymethyl 2 - Furaldehyde
+ Amines
+ Amines
MELANOIDINS
1-amino-1-deoxy-2-ketose (keto)
High PH
-H2NR
+ Amines
Reductones Dicarbonyls
Methyl Dicarbonyl Intermediate

Dehydration & Fragmentation



1- Amino -1- Deoxy-2-Ketose



3-Deoxyglycosulose

Dehydration

- Furfural
- HMF
- Pyranose
- Maltol

Fragmentation

Carbonyls & Dicarbonyls

- Pyruvaldehyde
- Diacetyl
- Acetone
- Dihydroxyacetone

Strecker Degradation Of Amino Acids

Amino Acid Dicarbonyl Compound Aldehyde

Significance of the Maillard Reaction in food processing :

Most prevalent – because it requires relatively **low energy of activation** and is **autocatalytic**.

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 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 to 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
Some of the compounds are known to be **carcinogenic** in laboratory animals.

5. Nutritional implications

Reduction in nutritive value.

- Loss of essential amino acids - especially **lysine**.
- Loss of some **vitamins**.
- Increase **excretion of Zn** in urine due to 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.

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.