water

1.1. Introduction

Water is relatively small inorganic molecule, but organic life is highly dependent on this tiny molecule. It is the only substance on the earth that occurs abundantly in all three physical states (gas, liquid and solid).

Water is essential for life: as:

(1) regulator of body temperature
(2) solvent
(3) carrier of nutrients and waste products
(4) reactant and reaction medium
(5) lubricant and plasticizer
(6) stabilizer of biopolymer conformation
(7) facilitator of the dynamic behavior of macromolecules (e.g. catalytic activity)

Most of the fresh foods contain large amounts of water. It is one of the major component in composition of many foods. Each food has its own characteristic amount of this component. Effect of water on structure, appearance and taste of foods as well as their susceptibility to spoilage depends on its amount, location, and orientation. Therefore, it is essential to know its physical properties.

Water has unusually high melting point, boiling point, surface tension, permittivity, heat capacity, and heat of phase transition values. Other unusual attribute of water include expansion upon solidification, large thermal conductivity compared to those of other liquids, moderately large thermal conductivity of ice compared to those of other nonmetallic solids.

1.2. Water Molecule

Some the unusual properties of water are due strong intermolecular attractive forces among molecules of water. The unusual properties of water can be explained from nature of water molecules. In formation of water molecule, two hydrogen atoms form covalent bonds with oxygen. The highly electronegative oxygen of the water molecule pulls the single electron from each of the two covalently bonded hydrogen atoms towards its self, as a result each hydrogen atom becomes partially positively charged and oxygen becomes partially negatively charged.
Consequently, resultant covalent bond formed between oxygen and hydrogen atoms acquires partial ionic character. The bond angle of individual water molecule in vapor state is 104.5°.

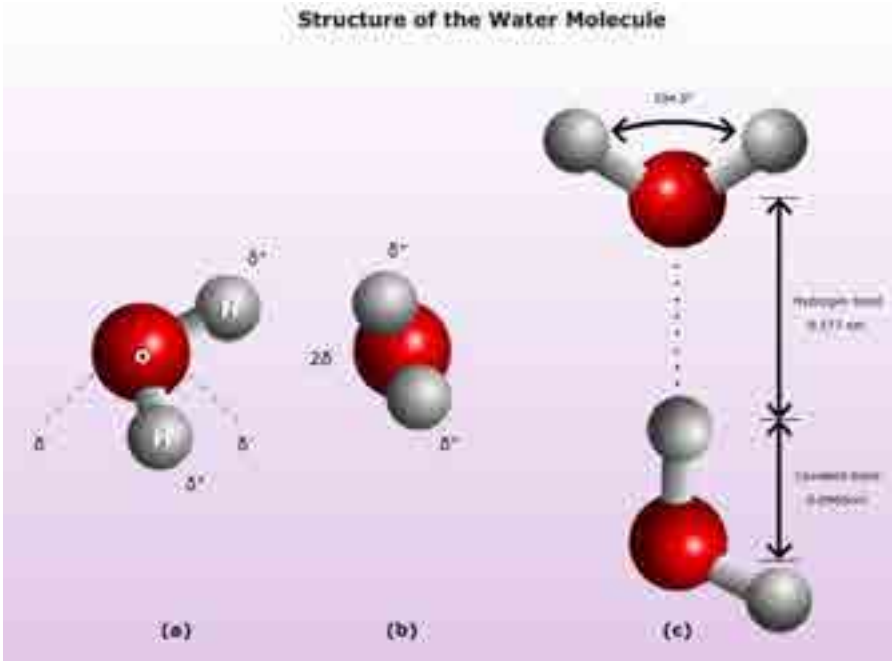


Figure 1.1 Structure of the water molecule

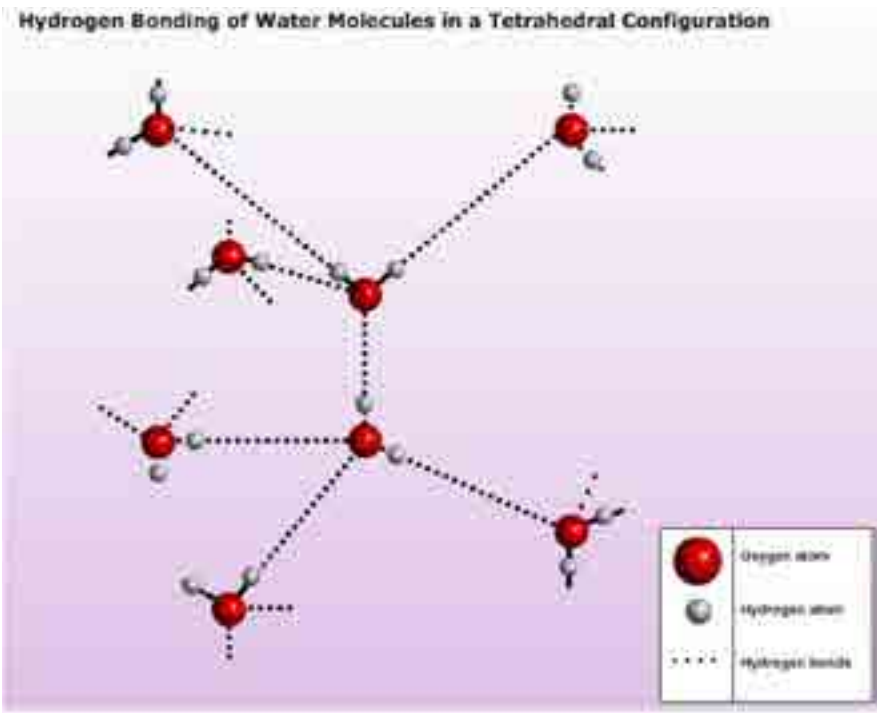


Figure 1.2 Hydrogen bonding of water molecules in a tetrahederal configuration

1.3. Association of Water Molecules

The shape of water molecule and the partial polar nature of the O-H bond in the water molecule create intermolecular attraction force. Such inter molecular attraction, results in to formation of hydrogen bonds between the water molecules. Therefore, water molecules associate with considerable tenacity.

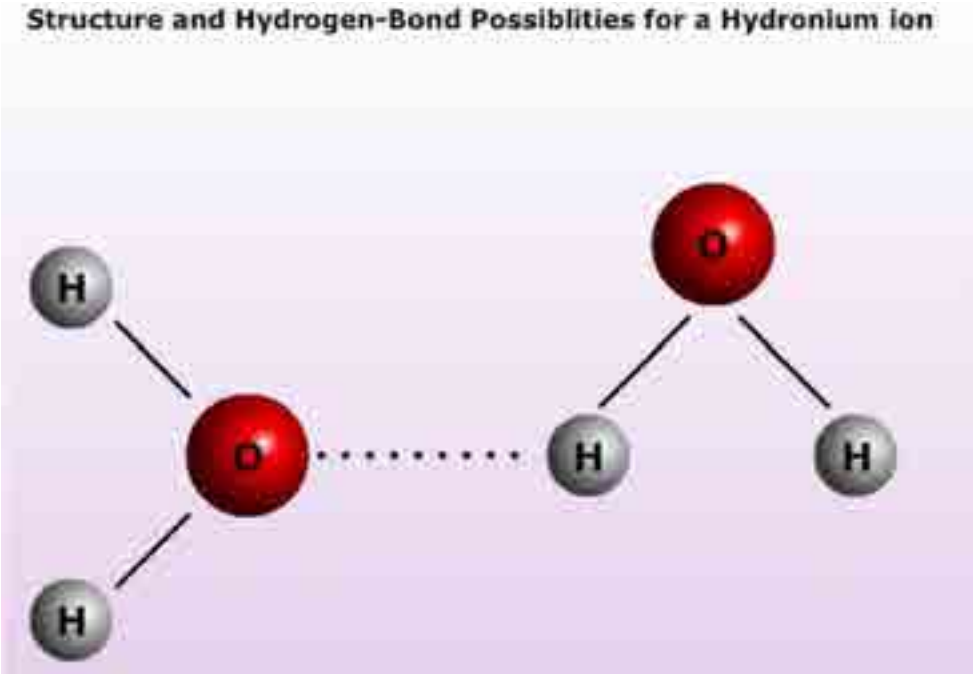


Figure 1.3 Hydronium ion

Each water molecule involves in four hydrogen bonds with neighboring water molecules. Multiple hydrogen bonding between water molecules, forms a structure of three-dimensional network.

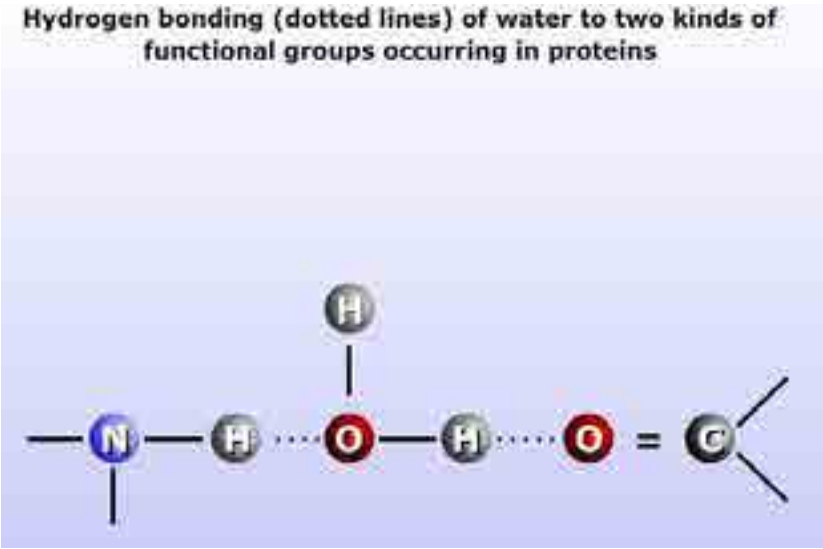


Figure 1.4 Hydrogen bonding of water to two kinds of functional groups occurring in proteins

Existence of three-dimensional hydrogen bonded structure of water is responsible for many of its unusual properties. The extra energy needed to break intermolecular hydrogen bonds. This leads to large values for heat capacity, melting point, boiling point, surface tension, and enthalpies of various phase transitions of water. The dielectric constant (permittivity) of water is influenced by hydrogen bonding. Hydrogen-bonded multi-molecular dipoles increase the permittivity of water. The hydrogen bonded arrangement of water molecules is highly dynamic, allowing individual molecules to alter their hydrogen-bonding relationships with neighboring molecules. This phenomenon facilitates mobility and fluidity of water.

The open, hydrogen-bonded, tetrahedral structure of water molecules in ice is responsible for low density of water in ice form. The extent of intermolecular hydrogen bonding among water molecules depends on temperature.

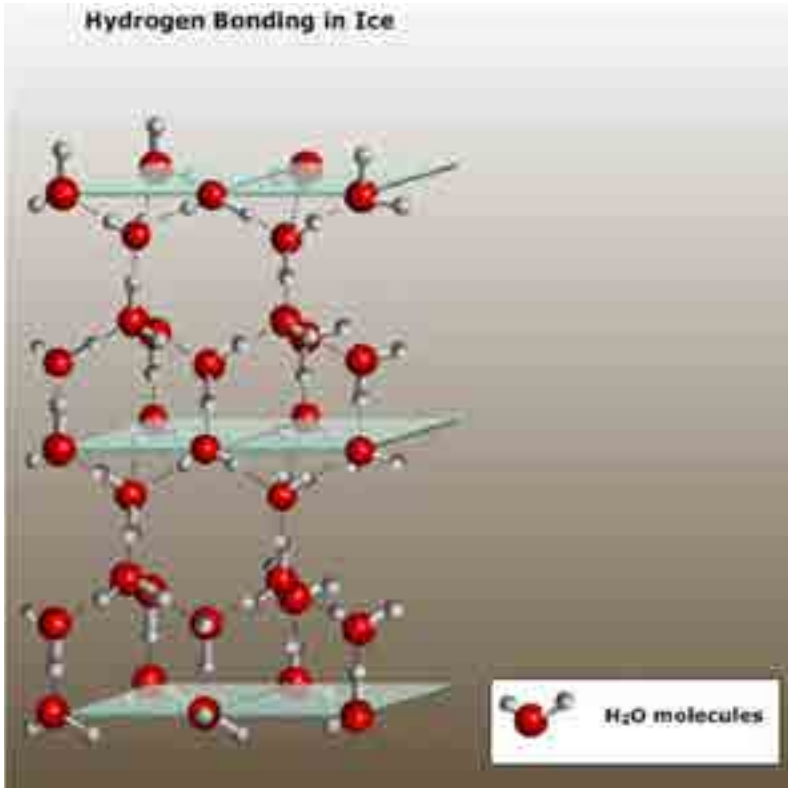


Figure 1.5 Hydrogen bonding in ice

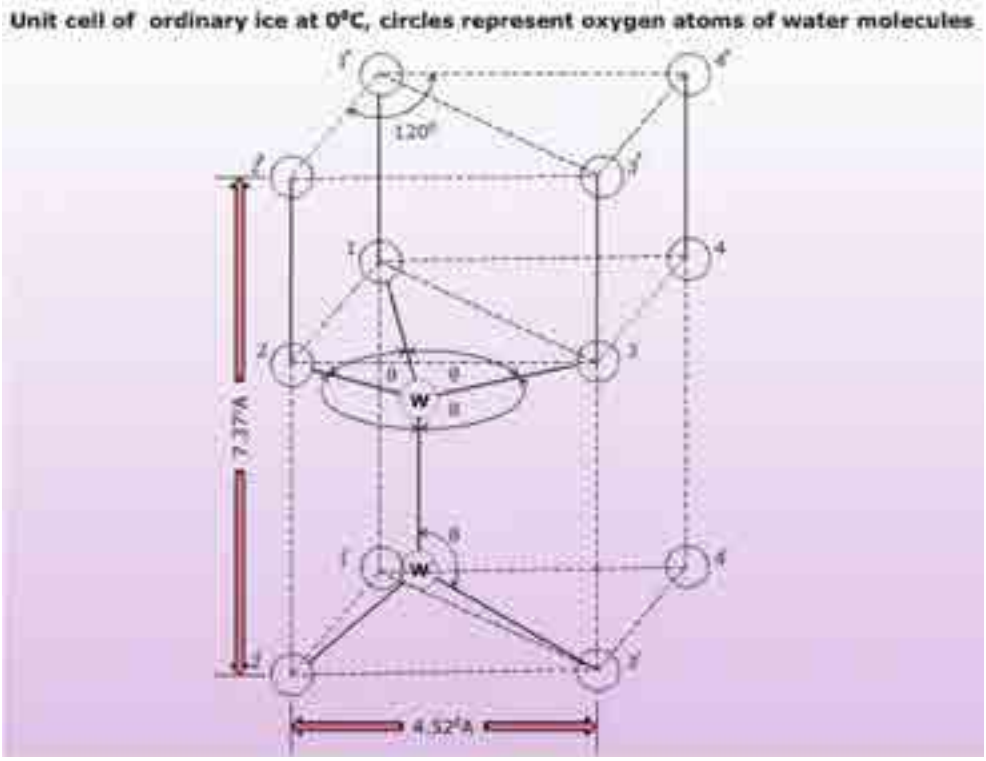


Figure 1.6 Unit cell of ordinary ice

With input of heat melting of ice occurs; that is, some hydrogen bonds are broken distance between nearest neighbor increases. The latter factor predominates at temperatures between 0 and 4°C, which causes net increase in density. Further warming increasing distance between nearest neighbors (thermal expansion) predominates above 4°C, which causes net decrease in density.

Water binding and chemical reactions mediated by water

2.1. Introduction

Mixing of solutes and water alters properties of each other. Hydrophilic solutes cause changes in structure and mobility of water and water causes changes in the reactivity, and structure, of hydrophilic solutes. Hydrophobic groups of solutes interact only weakly with water. In interaction of solute with water, various bonding forces existing between water and solutes.

To understand interaction between water and solutes at the molecular level, it is essential to knowledge about water-related phenomena and related terms like water binding, hydration, and water holding capacity. The terms “water binding” and “hydration” are often used to represent tendency of water to associate with hydrophilic substances in foods. The extent and tenacity of water binding or hydration depends on several factors like nature of solute, salt composition, pH, and temperature.

2.2. Water holding capacity

Term generally used to describe ability of a matrix of molecules to physically entrap large amounts of water in such a way that prevents exudation of the water. The food matrices that entrap water in this manner include pectin and starch gels and tissue cells of plant and animal. This physically entrapped water does not flow from food even when they are cut or minced. But this water behaves almost like pure water during food processing operations like drying, freezing, etc. it is also available as a solvent. Thus, bulk flow of this water is restricted, but movement of individual molecules almost remains same as that of water molecules in a dilute solution.

Impairment in this entrapment of water (*i.e.* holding capacity) of foods has a significant effect on quality of food. Some of the typical examples are oozing out of liquid from gel (syneresis) and exudation of liquid on thawing of frozen foods.

2.3. Bound Water

Bound water is not a easily identifiable entity. It is poorly understood term. Number of definitions proposed. The bound water is that water which

- is in equilibrium water of sample at appropriate temperature and relative
- humidity does not contribute significantly to permittivity and has restricted mobility
- does not freeze at low temperature (*e.g.* 40°C)
- unavailable as a solvent to dissolve additional
- solutes migrate with a macromolecule during sedimentation or flow

The bound exists in vicinity of solutes molecules. Properties of this water are significantly different from that of the “bulk” water in the same system. In high water content foods, the bound water account for very minute amount of the total water present. Generally, the first layer of water molecules adjacent to hydrophilic groups comprises the bound water.

2.4. Interaction between water and ions

Ions and ionic groups of organic molecules hinder mobility of water molecules to a greater extent than other types of solutes. The strength of water-ion interaction is greater than that of hydrogen bonds, between the water molecules, however, it is much less than that of covalent bonds. Water and inorganic ions (*e.g.* NaCl) undergo dipole-ion interactions.

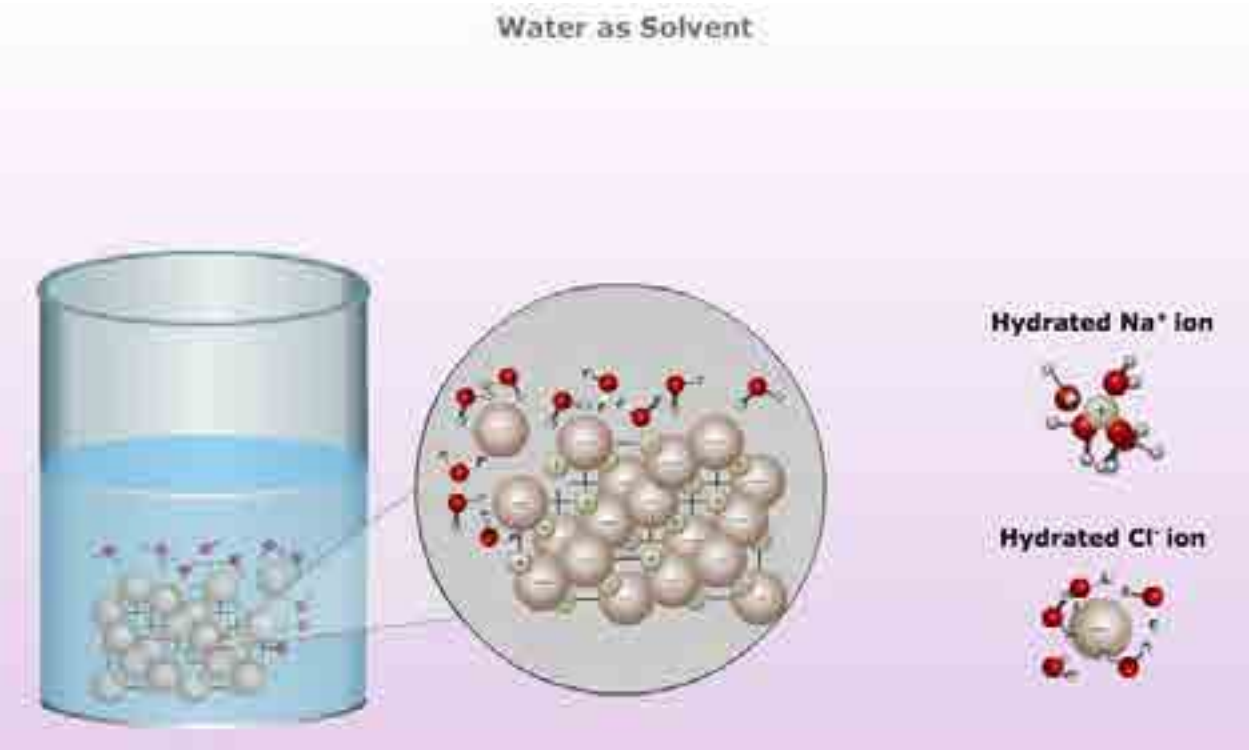


Figure 2.1 Water as solvent

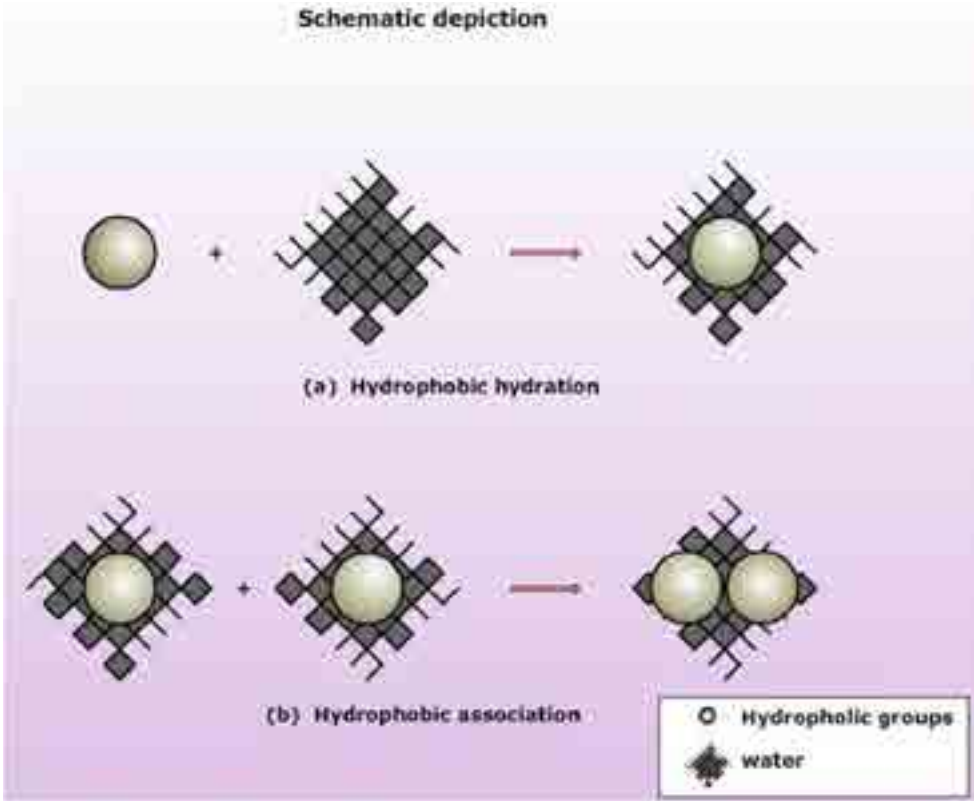
The ions compete for water and alter water structure, influence the permittivity of the aqueous medium and influence thickness of the electric layer around colloids particle "degree of hospitality" provided to other nonaqueous solutes and to substances suspended in the medium. Thus, conformation of proteins and stability of colloids are profoundly influenced by nature and concentration of ions present in the system. Salting-in and salting-out of protein are the important examples of such effect of ions.

2.5. Interaction between water and hydrophilic solutes forming hydrogen bond

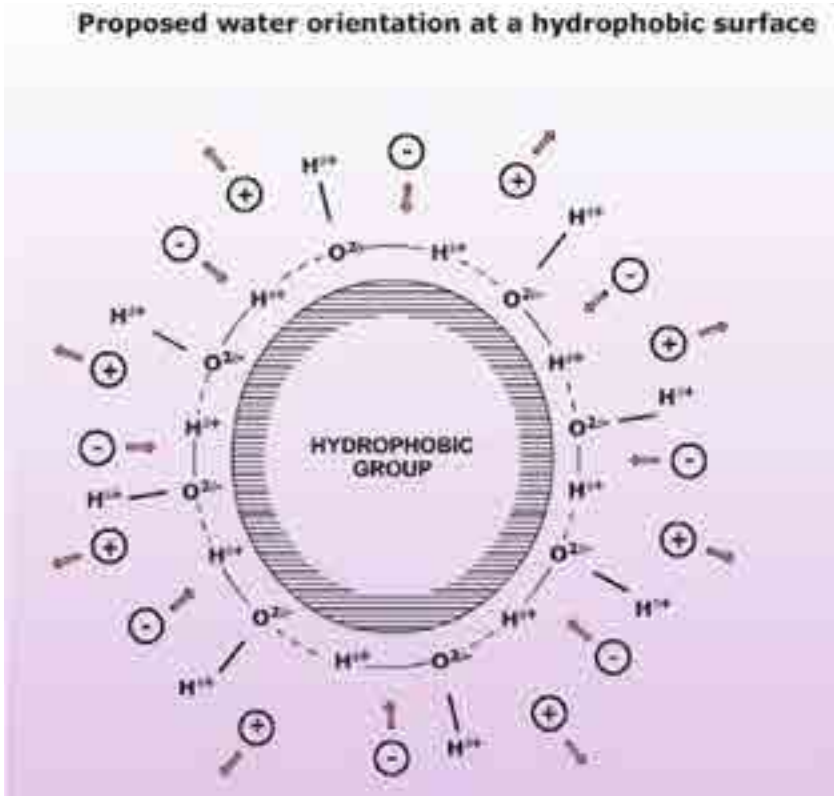
Interactions between water and nonionic, hydrophilic solutes are weaker than that of the interactions between water and ions and of the almost same strength as that of the hydrogen bonds between water molecules. Solutes capable of hydrogen bonding enhance or at least not disrupt the normal structure of pure water. However, in some instances solutes have a disruptive influence on the normal structure of water. Urea is good example which markedly disrupts normal structure of water.

2.6. Interaction between water and non-polar substances

The mixing of water and hydrophobic substances (*e.g.* apolar groups of fatty acids, amino acids, proteins, etc.) is thermodynamically unfavorable event ($\Delta G>0$). Water forms a special structure in vicinity of the incompatible apolar entities. This process has been referred to as hydrophobic hydration. Since hydrophobic hydration is thermodynamically unfavorable, water tends to minimize its association with the apolar entities. Therefore, the incompatible aqueous environment will encourage two separate apolar groups to associate, to decrease waterapolar interfacial area. This process is termed as "hydrophobic interaction".



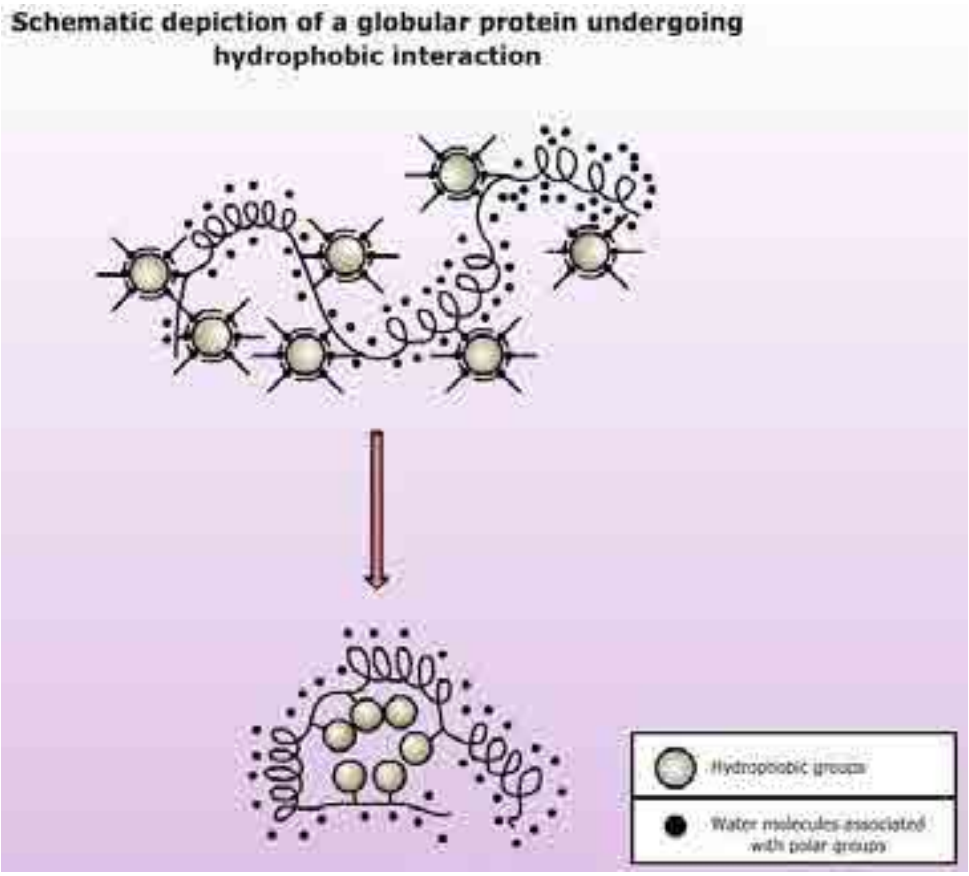
(Figure 2.2 Hydrophobic interaction)



(Figure 2.3 Water orientation at hydrophobic surface)

A clathrate hydrate is a cage-like structure inclusion compound, in which hydrogen-bonded water layer entraps a small apolar molecule. Formation of clathrate hydrates is an extraordinary ability of water to minimize contact with hydrophobic groups. This structure influences conformation, reactivity, and

stability of molecules like proteins. Hydrophobic interaction is of primary importance in maintaining the tertiary structure of most proteins. It provides a major driving force for protein folding, causing many hydrophobic residues to assume positions in the protein interior. Such association of water with hydrophobic groups of proteins has an important influence on functionality of the protein.



(Figure 2.4 Globular protein undergoing hydrophobic interaction)

The non-polar groups of other compounds such as alcohols, fatty acids, and free amino acids also can participate in hydrophobic interactions. Therefore, association of water with hydrophobic groups in proteins is very important in food. Reduction in temperature causes hydrophobic interactions to become weaker and hydrogen bounds to become stronger.

2.7. Water Activity

A definite relationship exists between water content of food and its perishability. Concentration and dehydration of food is carried out primarily to decrease its water content, with a view to increase concentration of solutes and thereby increase shelf life of the food. However, various foods with same amount of water content may differ significantly in perishability, which indicates that the water content

alone is not a reliable indicator for susceptibility of food towards perishability. This is largely due to differences in intensity of association of the food constituents with water molecules. Water having strong associations with food constituents has lower ability to support deteriorative activities like microbial growth and chemical degradation reactions (*e.g.*hydrolysis), than that of the weakly associated water. Consequently, term water activity (a_w) was developed to account for the intensity with which water associates with various nonaqueous constituents. Food stability, safety, and other properties can be predicted far more reliably from a_w than from water content. The term "activity" was derived from laws of equilibrium thermodynamics by G. N. Lewis and its application to foods was pioneered by Scott.

2.7.1. Definition

Water activity may be defined as ratio of tendency of a solvent to escape from solution (f_0) to tendency of the solvent to escape from pure solvent (f). At ambient pressure, f/f_0 is almost equal to relative vapour pressure of the solution. Therefore, a_w may also be defined as ratio of relative vapour pressure of solvent upon dissolving nonvolatile solute to the vapour pressure of pure solvent. Therefore, relative vapor pressure is also used interchangeably for a_w. The relative vapour is related to per cent equilibrium relative humidity (ERH) of the product environment.

Classification and physicochemical properties

4.1 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.

4.2. Classification of Proteins

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

4.2.1. 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.

Table-4.1: 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.

4.2.2. 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 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.

4.2.3. 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.

4.3. Physicochemical properties of proteins

4.3.1. 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.

4.3.2. 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.

4.3.3. 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.

4.3.4. 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.

4.3.5. 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.

4.3.6. 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.

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.

4.3.8. 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).

4.3.9. 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

5.1. 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.

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 (60º-90ºC/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^{o}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^{o}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^{o}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.

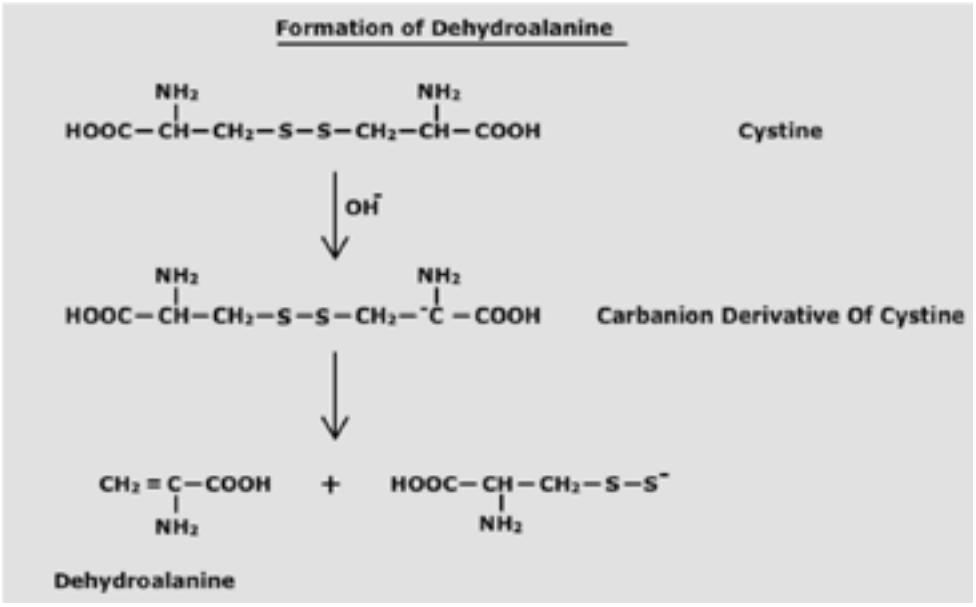
5.2.4. Racemization: Severe heat treatment at temperatures above 200^{o}C as well as heat treatment at alkaline pH (e.g. in texturized foods) invariably leads to partial racemization of L-amino acid residues to D-amino acid residues. Some racemization is also observed during acid hydrolysis of proteins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

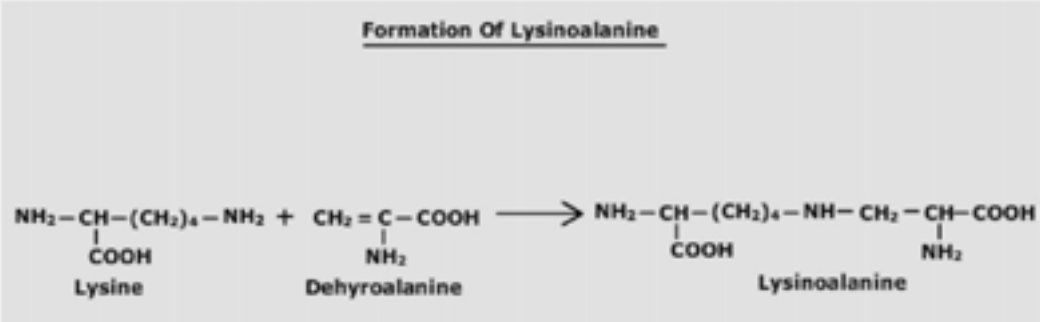
5.2.5. Effect of heat treatment at alkaline pH: Alkali treatment causes many reactions (undesirable reactions). The more common ones are hydrolysis, elimination reactions involving side chains of certain amino acids, racemization of amino acid residues, addition of compound to the proteins, scission 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^{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.

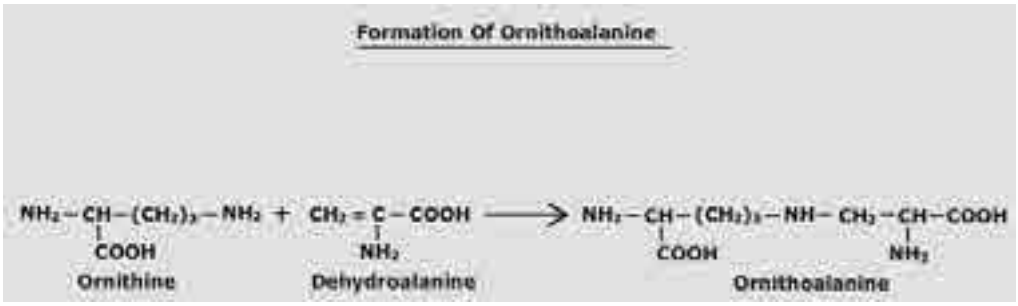
Formation of protein-protein cross-links in alkali treated proteins decreases their digestibility and biological value. Decrease in digestibility is related to the inability of trypsin to cleave the peptide bond in lysinoalanine. Cross-links also impose steric constraints that prevent the hydrolysis of other peptide bonds in the neighborhood of such cross links.



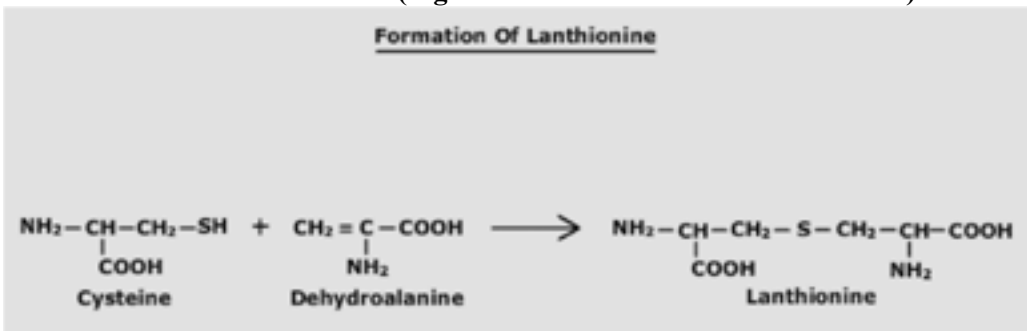
(Figure 5.1 Formation of Dehydroalanine)



(Figure 5.2 Formation of Lysinoalanine)



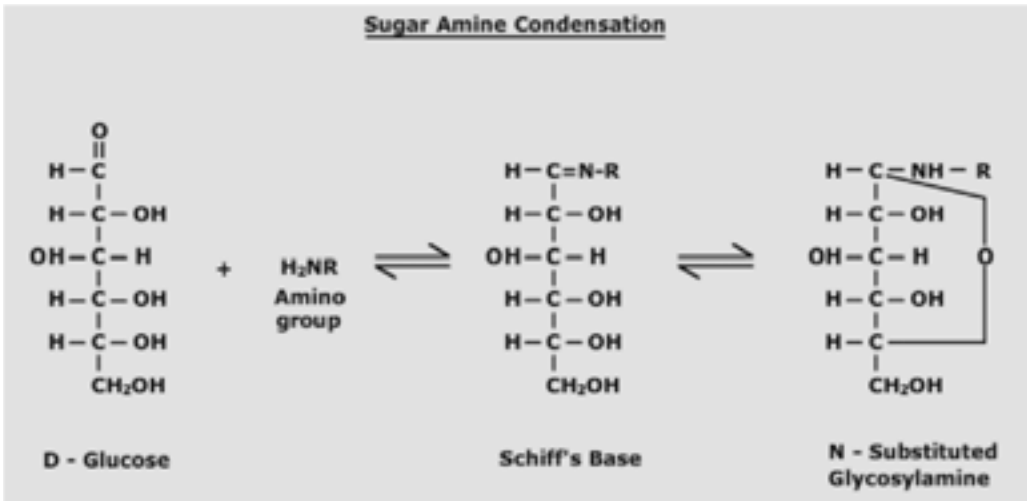
(Figure 5.3 Formation of Ornithoalanine)



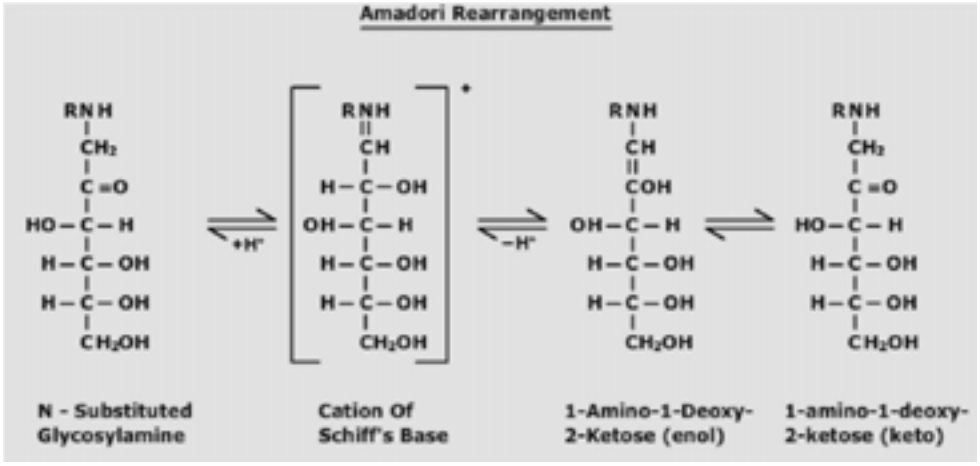
(Figure 5.4 Formation of Lanthionine)

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

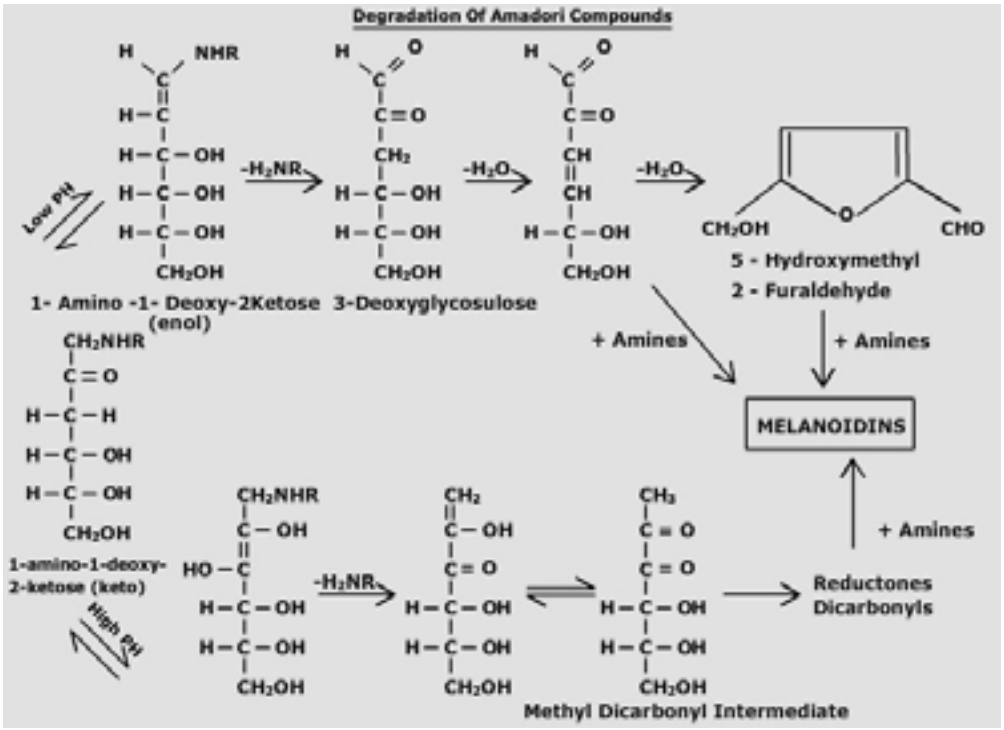
Maillard reaction (nonenzymic browning) refers to a complex set of reactions initiated by reaction between amines and carbonyl compounds, which, at elevated temperatures, decompose and eventually condense into insoluble brown products known as melanoidins. This reaction occurs not only in foods during 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.



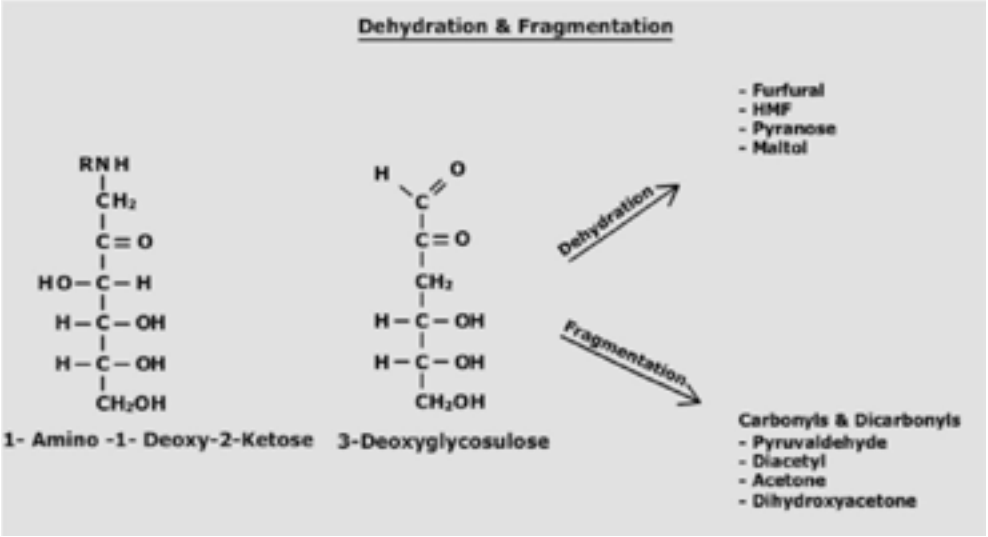
(Figure 5.5 Sugar Amine Condensation)



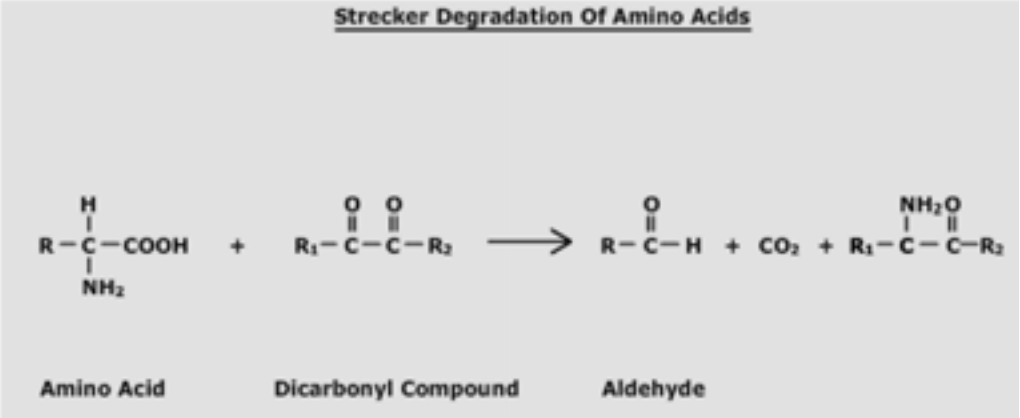
(Figure 5.6 Amadori Rearrangement)



(Figure 5.7 Degradation of Amadori Compounds)



(Figure 5.8 Dehydration and Fragmentation)



(Figure 5.9 Strecker Degradation of Amino Acids)

5.2.7 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.

5.2.8. 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.

Enzyme

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 step wise 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, the formation 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, enzymaticstage, the enzyme acts on κ-casein (hydrolysis of peptide bond between Phe_{105}-Met_{106})resulting in the formation of insoluble para-κcasein and a soluble glyco macropeptide. 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 proteinases are produced by a great number of bacteria and fungi. Chymotrypsinand 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 aremostly 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. amino peptidases,

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 solublization of denatured proteins
4. For maintenance of protein solubility in acid media
5. Increasing digestibility
6. Decomposition of those proteins that possess undesirable properties

fats and oils - classification and chemical composition

8.1. INTRODUCTION

Lipids are a broad group of naturally occurring molecules which includes fats, waxes, sterols, fat-soluble vitamins (A, D, E and K), triglycerides, diglycerides, monoglycerides, phospholipids, and others. Lipids are formed from structural units with a pronounced hydrophobicity. This solubility characteristic, rather than a common structural feature, is unique for this class of compounds. Lipids are soluble in organic solvents but not in water.

Fats and oils may be obtained from vegetables (various vegetable oils, cocoa butter, etc.), animal source (milk fat, lard, tallow, etc.) and marine (whale oil, cod liver oil, etc.). They play an important role in nutrition as well as physiological functions as they are rich energy source (9 kcal/g) and as a source of essential fatty acids and fat soluble vitamins. Some lipids are amphiphilic in nature (contain both hydrophilic & hydrophobic groups) with surface-active properties. As a whole, fats enrich the nutritional quality and impart the desired body & texture, rich mouth feel to the food. It also contributes characteristic flavour to food and produces a feeling of satiety or loss of hunger.

8.2. CLASSIFICATION OF LIPIDS

Lipids are classified on several basis, i.e. based on its complexity, ability to react with alkali (saponification process) to form soap and polarity (charge on its components).

BASED ON STRUCTURE/COMPLEXITY: Based on structure, lipids can be classified into three groups, i.e. simple, complex and derived lipids.

Simple lipids: These lipids are composed of fatty acids and alcohol components, and include fats, oils and wax esters. They can be hydrolyzed to two different components, usually an alcohol and an acid.

Compound lipids: These lipids include glycerophospholipids (phospholipids), glyceroglycolipids (glycolipids), and sphingolipids. On hydrolysis, it yields three or more different compounds.

Derived lipids: They meet the definition of a lipid but are not simple or compound lipids and include fatty acids and alcohols; which are the building blocks for the simple and complex lipids. It also includes sterols, vitamins, pigments, hydrocarbons, etc.

BASED ON POLARITY

Based on polarity lipids are classified into two groups, i.e. polar lipids and non-polar lipids.

Polar lipids

They are charged molecules

Soluble in polar solvents like alcohol, acetone, etc.

e.g. phospholipids, glyceroglycolipids, fatty acids, etc.

Non-polar lipids

They are uncharged molecules

Soluble in non-polar solvents like ether, benzene, hexane, etc.

e.g. Glycerides, sterols, sterol esters, Carotenoids, waxes, vitamins, etc.

BASED ON SAPONIFICATION

It is classified based on the ability of lipids to react with alkali (saponification process) to form soap. Based on this reaction, lipids are grouped as saponifiable lipids and unsaponifiable lipids.

Saponifiable lipids

React with alkali and form soap

Present in large amount

e.g. Glycerides, phospholipids, fatty acids, cholesterol ester, etc.

Unsaponifiable lipids

Do not react with alkali to form soap

Present in relatively small amount

e.g. Fat soluble vitamins, sterols, hydrocarbons, carbonyls, etc.

8.3. TRADITIONAL CLASSIFICATION OF EDIBLE FATS/OILS

It is classified based on the source of fat/oil and the constituent fatty acids present.

Milk fat

They are derived from milk of mammals, particularly from buffalo, cow, goat and sheep.

Major fatty acids of milk fat are palmitic ($C_{16:0}$), stearic ($C_{18:0}$) & oleic ($C_{18:1}$) acids.

Contains appreciable amounts of short chain fatty acids ($C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$).

Butyric acid ($C_{4:0}$) is a characteristic fatty acid to milk fat.

Lauryl or Lauric acid fat

Characteristic fatty acid is Lauric acid (Almost 40 - 50 % of total fatty acids).

Low amount of unsaturated fatty acids and thus having low melting point.

Contain moderate amount of $C_{6:0}$, $C_{8:0,}$ $C_{10:0}$ fatty acids.

Obtained from certain species of palm, ex. Coconut.

Vegetable butters

Obtained from the seeds of various tropical trees, ex cocoa.

Characterized by their narrow melting range, i.e. due to arrangement of fatty acids in the triglyceraldehyde molecules.

Widely used in the manufacture of confectionary products, ex. Chocolates, etc.

Oleic –linoleic acid fats

Fat present in this group are the most abundant and of vegetable origin.

Contain large amounts of oleic and linoleic acids

Contain less amount of saturated fatty acids (i.e. less than 20%)

Cottonseed, corn, peanut, sunflower, palm olive and sesame oils are important examples.

Linolenic acid fats

Contain large amount of linolenic acid.

Soybean, rapeseed, wheat germ, hempseed, etc. with soybean being the most important.

Linolenic acid in soybean oil is responsible for off-flavour, i.e. flavour reversion problem.

Animal body fats

It is known as depot fats from domestic land animals e.g. lard and tallow.

Contain large amounts of C_{16}>C_{18} fatty acids

Contain medium amounts of unsaturated fatty acids (mostly $C_{18:1}$> $C_{18:2}$)

Contain appreciable amounts of saturated triacylglycerols and shows high melting points.

Egg lipids are important due to their emulsifying properties and high content of cholesterol.

Marine oils

Contain large amounts of omega-3-polyunsaturated fatty acids, with up to six double bonds Usually rich in vitamins A & D.

8.4. CHEMICAL COMPOSITION

Table-8.1. Gross chemical composition of fats of various species

Sr. No.	Class of lipids	Cow milk fat	Buffalo milk fat	Human milk fat
		(% weight basis)		
1.	Triacylglycerols	97.5	98.6	98.2
2.	Diacylglycerols	0.36	0.4	0.7
3.	Monoacylglycerols	0.02	0.03	traces
4.	Cholesterol	0.31	0.3	0.25
5.	Cholesterol Esters	traces	0.1	traces
6.	Phospholipids	0.6	0.5	0.26
7.	Free fatty acids	0.027	0.5	0.4

8.5. UNSAPONIFIABLE MATTER OF VARIOUS FATS AND OILS

All the fats and oils have a tendency to form soap when they are allowed to react with alkali. This reaction is called saponification reaction. It is defined as "The number of milligrams of KOH required to saponify one gram of fat". The saponification value (SV) is related to molecular weight of the constituent fatty acids in a particular fat. Fats and oils contain an average of 0.2-1.5% unsaponifiable compounds. The reaction involved in saponification process is shown below.

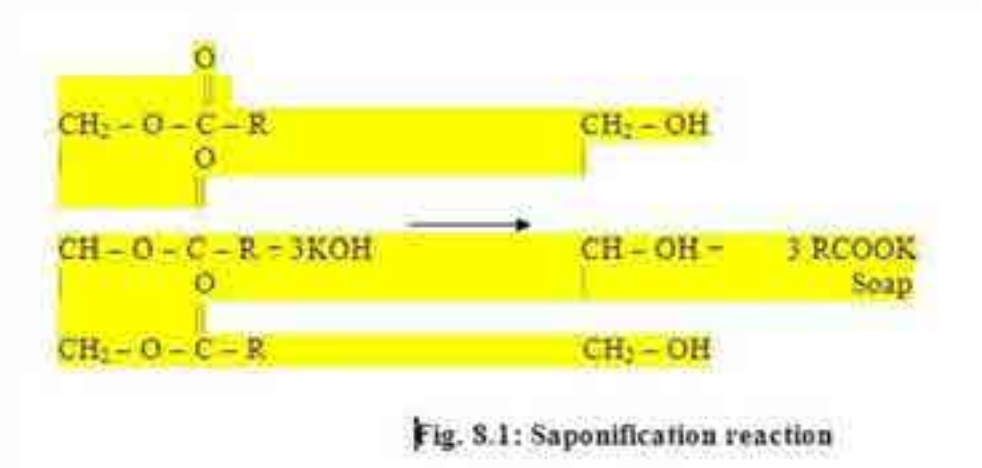


Fig. 8.1: Saponification reaction

The unsaponifiable fraction of fats consists of sterols, terpenic alcohols, aliphatic alcohols, squalene, and hydrocarbons. The composition of various components of unsaponifiable fraction in some fats and

oils is given in Table 8.2. In most fats the major components of the unsaponifiable fraction are sterols. Animal fats contain cholesterol whereas plant fats and oils contain phytosterols with no or only trace amounts of cholesterol. The predominant phytosterol is 3-sitosterol; the others are campesterol and stigmasterol. Sterols are compounds containing the perhydrocyclopenteno- phenanthrene nucleus, which they have in common with many other natural compounds, including bile acids, hormones, and vitamin D.

The sterols provide a method of distinguishing between animal and vegetable fats by means of their acetates.

Cholesterol acetate has a melting point of 114°C, whereas phytosterol acetates melt in the range of 126 to 137°C. This provides a way to detect adulteration of animal fats with vegetable fats. The various constituents present in unsaponifiable matter of lipids are discussed below:

a) Hydrocarbons: All edible oils contain hydrocarbons with an even/odd carbon number (C_{11}-C_{35}). Olive, rice and fish oils are particularly rich in this class of compounds. The main hydrocarbon constituents of olive oil (17g/kg) and rice oil (~3.3g/kg) is linear tri-terpene known as squalene (C_{30}). This compound is used as analytical indicator for olive oil. It is also present in substantially high amount in fish liver oil.

b) Sterols: Sterols are compounds containing perhydro cyclopenteno–phenanthrene tree nucleus. The steroid skeleton contains 4 condensed rings A, B, C and D. A characteristic in steroids is the presence of an alcoholic – OH group in position 3. In most fats, the major component of unsaponifiable fraction is that of the sterols. In animal fats, it is mainly cholesterol while in plant fats/oils it is phytosterol. The prominent phytosterol is βsitosterol. Cholesterols are obtained biosynthetically from squalene. In animals, cholesterol is the precursor for the biosynthesis of steroids and bile acids. Cholecalciferol (vitamin D3) is formed by the photolysis of 7-dehydro cholesterol.

The main steroid of yeast is ergosterol (pro-vitamin D2). This is converted by irradiation (UV) into ergocalciferol
(Vitamin D2)

c) Tocopherols and Tocotrienols: The methyl derivatives of tocol are denoted as tocopherols. Some methyl derivatives of Tocotrienols are also found in foods. Because α-tocopherol is the most abundant tocopherol and it appears to have the greatest biological activity, so, α -tocopherol content of foods is usually considered to be the most important. These redox type lipids are important as antioxidants in foods containing fats and oils.

d) Carotenoids: They are polyene hydrocarbons biosynthesized from 8 isoprene units and have 40 carbons. They provide the intensive yellow, orange or red colour to a great number of foods of plant origin. They are synthesized only by plants. However, they reach animal tissues via the feed and can be modified and deposited there.

Carotenoids are divided into two classes:

Carotenes: carotenes are pure polyene hydrocarbons.

Xanthophylls: Xanthophylls have oxygen in the form of hydroxy, epoxy or oxo groups and are present in corn, green leaves, egg yolk, etc.

Table- 8.2: Composition of the Unsaponifiable matter of some Fats and Oils

Fats/Oils	Hydrocarbons	Squalene	Aliphatic Alcohols	Terpenic Alcohols	Sterols
Olive	2.8-3.5	32-50	0.5	20-26	20-30
Linseed	3.7-14.0	1.0-3.9	2.5-5.9	29-30	34.5-52
Teaseed	3.4	2.6	-	-	22.7
Soybean	3.8	2.5	4.9	23.2	58.4
Rapeseed	8.7	4.3	7.2	9.2	63.6
Corn	1.4	2.2	5.0	6.7	81.3
Lard	23.8	4.6	2.1	7.1	47.0
Tallow	11.8	1.2	2.4	5.5	64.0

Reactions involved during deep frying of food

9.1. INTRODUCTION

Deep frying is one of the methods of food preparation used both in the home and in industry.

Several food products like potato chips, meat, fish, etc prepared by frying it into fat/oil heated to about 180 °C. After some time of frying process, the food article is sufficiently cooked to be consumed. Due to prolonged heating at very high temperature, substantial changes take place in chemical and physical properties fat or oil. The changes taking place in fat/oil during frying affects the quality of fat/oil being used and quality of finished foods. When period of frying is short, the changes are mostly desirable as there is improvement in the organoleptic quality of product, due to production of desirable flavour and aroma. During such short periods characteristics and concentration of undesirable compounds originating due to heating do not cause any problem. In continuous deep fat frying, large quantities of fat are absorbed by food. This has to be replenished by fresh frying oil. This replenishment results in a steady state condition wherein it is unlikely that the oil deteriorates beyond a certain point. In intermittent frying, fats remain hot for long periods and undergo many heating and cooling cycles before they are used up by subsequent frying operations. This results in more rapid destruction of fat probably due to increase in hydroperoxides upon cooling, followed by their decomposition when fat is reheated.

9.2. BEHAVIOUR OF FRYING OIL

Different classes of compounds produced from oil during deep fat frying. These compounds are given below:

9.2.1. Volatiles

Oxidative reactions involving formation and decomposition of hydroperoxides lead to production of Saturated and unsaturated aldehydes, ketones, hydrocarbons, lactones, alcohols, acids and esters

The amounts of volatiles produced vary widely depending on type of oil, type of food and extent of heat treatment

Generally reaches plateau values because balance achieved between formation of volatiles and their loss due to evaporation and/or decomposition

9.2.2. Nonpolymeric polar compounds of moderate volatility

e.g. hydroxyl and epoxy acids produced through oxidative pathways

9.2.3. Dimeric and polymeric acids and glycerides

Occur from thermal and oxidative free radicals through polymerization of the radicals which results in a substantial increase in viscosity of the frying oil

9.2.4. Free fatty acids

Arise from hydrolysis of triacylglycerol in presence of heat and water
These reactions are responsible for various physical and chemical changes in the frying oil

Increase in viscosity and foaming tendency

Changes in colour (dark) and flavor

Decrease in iodine value and surface tension

Changes in refractive indices

9.2.5. Behaviour of food during frying (Event occur during frying of food)

Water is continuously released from the food into hot oil. This produces a steam distillation effect, sweeping volatile oxidative products from the oil. The released moisture agitates the oil and hastens hydrolysis. Blanket of steam formed above the surface of the oil tends to reduce the amount of oxygen available for oxidation Volatiles may develop in food itself and/or from the interactions between food and oil.

Food absorbs varying amounts of oil during deep fat frying

Sizable amounts of oil/fat are carried with the food – 5 to 40% by weight, e.g. potato chips have a final fat content of about 35% resulting in need for addition of fresh oil

Food itself can release some of its endogenous lipids into frying oil/fat

e.g. fat from chicken

consequently oxidative stability of new mixture may be different from that of the original frying oil/fat Presence of food causes the oil/fat to darken at an accelerated rate.

9.3.CHANGES IN FRYING MEDIUM

Hydrolysis, oxidation and polymerization are due to the chemical reactions that take place during deep fat frying.

Factors influencing the proportions of breakdown components in vegetable oils are:

Temperature	Method of heat transfer
Presence of O_2	Metals in contact with oil
Heating time	Turnover
Frying capacity	Nature of food being fried

The various chemical changes commonly observed are:

9.4.OXIDATION AND DECOMPOSITION

Release of moisture, high temperature and exposure to atmospheric O_2 during frying of fats favours the oxidation of frying medium. As food enters oil, oxygen is introduced into the oil leading to oxidative changes. After an initial induction period, the peroxide content of food begins to increase and finally decreases. The major reactions occurring during the autoxidation include degradation reactions resulting in the formation of volatile compounds. Autoxidation of unsaturated fatty acids leads to the formation of conjugated hydroperoxides and peroxides, which decompose to form volatile aldehydes, ketones, acids, alcohols and hydrocarbons.

9.5.THERMAL OXIDATION

The process of thermal oxidation also occurs when oil is heated at high temperature in the presence of O_2.

Thermal oxidation results in:

Formation of free fatty acids due to cleavage and oxidation of double bonds

Formation of hydroperoxides which may undergo-

Fission to form alcohol, aldehydes and acids, which contribute to darkening of frying medium and flavour changes.

Dehydration to form ketones

Formation of free radicals followed by their combination to form dimers, trimers, epoxides, alcohols and hydrocarbons, all of which contribute to increase in the viscosity of the oil.

During deep fat frying, thermal and oxidative decomposition of oil produces volatile and non-volatile products.

9.5.1. Volatile decomposition products: Most of them are removed by steam generated during frying. They contribute to flavour of deep fried products, e.g. unsaturated lactones

9.5.2. Non volatile decomposition products: These are formed largely due to thermal oxidation and polymerization of unsaturated fatty acids present in frying medium. These products include polymeric triglycerides, cyclic acids, fatty acids and other oxidative products. The accumulation of these products is responsible for changes viz. increase in FFA content, carbonyl value, -OH content and saponification value and decrease in unsaturation with resultant decrease in Iodine value. Such changes are also accompanied by increase in viscosity and refractive index.

9.6. POLYMERIZATION

The oxidation and thermal alteration products undergo polymerization forming gums and residues. Reactions between fatty acids of same/different triglycerides form cyclic and non-cyclic dimers and other polymeric compounds involving C-C linkages and oxygen bonding. Thermal polymerization of unsaturated fatty acids also yields cyclic monomers, dimers, trimers and higher polymers. The rate of polymerization increases with increase in unsaturation of triglycerides and frying time. This results on changes in molecular weight, viscosity, heat transfer rate, foaming, darkening of colour and gum accumulation. Polymerization also causes increased absorption of fat by food making it unpalatable and greasy.

9.7. HYDROLYSIS

Moisture that is continuously released from the food during frying brings about hydrolysis of fat causing an increase in acidity, due to the initial formation of FFAs, mono and diglycerides and glycerols; Soaps of some fatty acids are also formed which accelerate the deterioration of frying medium. Accumulation of alkaline material decreases the interfacial tension between the product and frying medium and decreases the food quality. Liberation of the FFAs causes a decrease in smoke point of oil. Viscosity, colour and iodine value of hydrogenated oils changes more rapidly at FFA levels of ~1.5%.

Polysaccharide - linear, branched and modified

11.1. Introduction

The carbohydrates constitute one of nature's three most abundant classes of organic compounds, the other two being the fats and the proteins. Carbohydrates are essentially substances that are made up of carbon, hydrogen and oxygen only. The carbohydrates are divided into three broad categories, namely, monosaccharides, oligosaccharides and polysaccharides. Monosaccharides represent the group of carbohydrates that cannot be further hydrolyzed to smaller molecules. They form the building blocks of the more complex carbohydrates. Oligosaccharides comprise the low molecular weight polymers that include the disaccharides and trisaccharides and compounds with as many as ten monosaccharides linked into single molecules.

11.2. Classification of carbohydrates

Carbohydrates may be broadly classified as follows:

I. Monosaccharides

1. Trioses, $C_3H_6O_3$, e.g. glyceraldehydes and dihydroxy acetone
2. Tetroses, $C_4H_8O_4$, e.g. erythrose, threose
3. Pentoses, $C_5H_{10}O_5$, e.g. arabinose, xylose, ribose, deoxyribose
4. Hexoses, $C_6H_{12}O_6$, e.g. glucose, galactose, fructose, mannose

II. Oligosaccharides

1. Disaccharides, $C_{12}H_{22}O_{11}$, e.g. sucrose, lactose, maltose
2. Trisaccharides, $C_{18}H_{32}O_{16}$, e.g. raffinose
3. Tetrasaccharides, $C_{24}H_{42}O_{21}$, e.g. Stachyose

III. Polysaccharides

1. Pentosans, e.g., araban, xylan
2. Hexosans, e.g., Starch, glycogen, cellulose, mannan, gallactan
3. Complex polysaccharides, e.g. hemicelluloses, gums, pectins

11.3 Polysaccharides

Polysaccharides are the carbohydrates which contain more than 10 monosaccharide units. They can be hydrolyzed into hundred or even thousands of monosaccharide units.

The suffix –ose in sugar is changed to –ans to describe the corresponding polysaccharide.

Examples:

(1) Pentosans - (a) Arabans

(b) Xylans

(2) Hexosans - (a) Glucans à starch, dextrin, glycogen, cellulose, inulin

(b) Mannans

(c) Galactans

(3) Complex polysaccharides

(a) Pectins or pectic substances

(b) Gums

(c) Mucilages

(d) Algal polysaccharides à Alginic acid and carrageenan.

(e) Bacterial polysaccharides à Xanthan gum.

11.4. Classification of polysaccharides

Polysaccharides occur in an infinite variety of different structural types which can be broadly classified into homopolysaccharides and heteropolysaccharides.

(1) **Homopolysaccharides**: They contain the same structural units throughout. For example, the glucans (starch and glycogen), fructans, mannans etc. These polymers can possess either simple linear structure or branched structures of varying complexity with more than one type of inter unit linkage.

Perfectly linear polysaccharides: They compounds with single neutral monosaccharides structural unit with only one type of linkage are denoted as perfectly linear polysaccharides. They are usually insoluble in water and can be solublized only under drastic conditions. They also have tendency to precipitate from solution retrogradation.

Branched polysaccharides: They are more soluble in water than linear polysaccharides as the chain-chain interaction are less pronounced. Compare to the linear polysaccharides of equal molecular weight and concentration, solution of branched polysaccharides have the lowest viscosity and lower tendency to precipitate.
They have the ability to form the sticky paste at higher concentration, probably due to side chain – side chain interaction. So they are suitable as binder or adhesives.

Linearly branched polysaccharides: They are polymers with long backbone chains and with mainly short chain viz. alkyl cellulose. They have properties, which are a combination of perfect linear and branched polymers. The long backbone chain is responsible for high viscosity of the solution. The presence of numerous short-side chains weakens the interaction between molecules and thereby gives it good solubility and rehydration rates and also provides stability to highly concentrated solutions.

Modified Polysaccharides: The properties of polysaccharides can be modified by physical and chemical methods that result in products suitable for specific purposes in the food industry. The solubility in water, viscosity and stability of solutions are all increased by binding neutral subsituents to linear polysaccharide chain e.g. hydroxypropyl cellulose. Binding acid groups (carboxymethyl, sulphate groups) also results in increased solubility and viscosity.

(2) Heteropolysaccharides

They contain two or more types of different monomer units. For example, the arabinoxylans, glucomannans etc. These biopolymers can be linear or branched to varying degrees with different types of branch points. Typical sugars units found in polysaccharides which occur in food are

a. D- Fructose (Fru)

b. D-mannose (Man)

c. D-Glucose (Glu)

d. D-Mannuronic acid (Man A)

e. D-Glucuronic acid (Glu A)

f. D-Xylose (Xyl)

g. D-Galactose (Gal A)

h. L-Arabinose (Ara)

i. D-Galacturonic acid (Gal A)

j. L-Rhamnose (Rha)

Table-11.1: Homopolysaccharides occurring or used in foodstuffs

Type	Linkage	Structure	Polysaccharide	Occurrence
Glucans	α, 1 → 4	Linear	amylose	Starchy material
	α, 1 → 4 α, 1 → 6	Branched	amylopectin	Starchy material
	α, 1 → 4 α, 1 → 6	Branched	glycogen	Animal liver
	β, 1→ 4	Linear	Cellulose	Cell walls of all plants
	β, 1→ 3 β, 1→ 4	Linear	β - glucan	Cereal grains (oats, barley)
Fructans	β, 2→ 6 β, 2→ 1	Branched	Fructans	Various plants (wheat endosperm)
	β, 2→ 1	Linear	Inulin	Jerusalem artichokes
Arabinans	α, 1 → 3 α, 1 → 5	Branched	Pectic substances	Sugar beet, citrus pectins
Xylans	β, 1→ 4	Linear	Xylans	Cell walls of plants

Table: 11.2-Heteropolysaccharides occurring or used in foodstuff

Units	Structure	Polysaccharide	Occurrence
Ara; Xyl	Branched	Arabinoxylans	Plant cell walls (wheat flour)
Glu A; Xyl	Branched	Glucuronoxylans	Plant cell walls
Glu; Man	Linear	Glucomannans	Seeds
Glu A ; Man A	Linear	Alginic acid	Brown seaweeds
Gal; Man	Branched	Guar/carbo gum	Leguminous seeds
Anhydro Gal; Gal sulphate	Linear	carrageenan	Brown seaweeds
Gal A; Rha	Linear	Pectic materials	All plant material
Ara; Rha; Gal; Glu A; Glu	Branched	Gum arabic	Trees (Acacia spp.)
Gal A; Xyl; Gal; Fuc	Branched	Gum tragacanth	Trees (Astragalu spp.)

Properties and Utilization of Common Polysaccharides – Cellulose, Glycogen, Hemicelluolose, Pectin,

Agar, Alginate, Carrageenan, Gums and Starch

12.1. INTRODUCTION

Polysaccharides are the carbohydrates which contain more than 10 monosaccharide units. They can be hydrolyzed into hundred or even thousands of monosaccharide units. Polysaccharides commonly present in foods are starch, glycogen, cellulose, hemicellulose, pectic substances, gums.

12.2. STARCH

Starch is a natural polymer of the sugar D-glucose. Starch occurs widely in the vegetables kingdom. The important of starch in food processing is based on the fact that it provides a very high proportion of the world's food energy intake; over 80 % of all food crops are composed of cereals and starchy – food crops.

Starch occurs in nature in the form of microscopically small, spherical particles or granules whose size and shape are characteristic for each species. The granules can be shown by ordinary and polarized light microscopy and by X-ray diffraction to have a highly order crystalline structure.

It is formed in plants by the condensation of a large number of glucose molecules (few hundred to several thousand units) into two types of polymers. One of these is a linear polymer, amylose, that is made up of more than 2000 glucose units. The individual glucose units are connected to each other by α-1,4-glycosidic linkage. A second starch component, called, amylopectin, has a highly branched structure, with each branch consisting of 20 to 30 glucose units, and each molecule containing several hundreds of these branches. The glucose units in each linear branch are connected by α-1,4 linkage. The branch points, are connected through α-1,6-glycosidic linkages. Both amylose and amylopectin molecules are deposited in starch granules in an orderly radial pattern.

The important sources of starch are

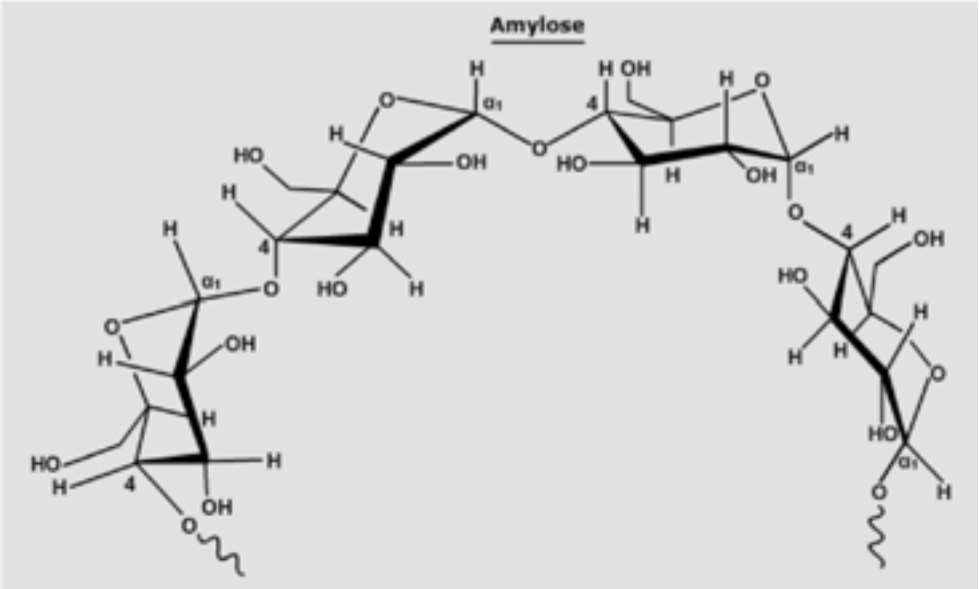
(i) Cereals and millets (65 to 85 %) e.g. maize, wheat, rice
(ii) Roots and tubes (19 to 35 %) e.g. potato, tapioca

Cereal starches differ from root and tuber starches in their physical properties. A cereal starch paste (5%) sets to a thick jelly on cooling whereas a turber starch paste (5%) remains as a fluid and does not set to a thick jelly.

In cereals moisture content is low and the starch granules are embedded in a hard, proteinaceous matrix, which requires preliminary softening before starch extraction. Potato contains high moisture and no preliminary softening is required.

12.2.1. Amylose

This is a long unbranched chain of D-glucose molecules linked together by α-1,4 linkage, similar to that present in maltose.



(Figure 12.1 Amylose)

Molecular weight of amylose range from 10^5 to 10^6 daltons and one molecule of amylose may contain 500 to 5000 glucose molecules. The solution on keeping turns turbid due to the precipitations of amylose by a process known as retrogradation. Amylose is mainly responsible for the stiffening of cooked rice on standing. Amylose gives a blue colour with iodine. Amylose content of a starch can vary considerably depending on the botanical species. Cereal starches such as wheat starch contains 25 – 30% amylose, corn starch (amylomaize) contains 40 – 80% amylose. Waxy maize contain 0% starch.

12.2.2. Amylopectin

Amylopectin is branched chain polysaccharide component of starch. In this polysaccharide short chains (20 to 30 molecules) of D- glucose linked by α-1,4 linkages. These chains are linked to each other by α-1,6 linkages.

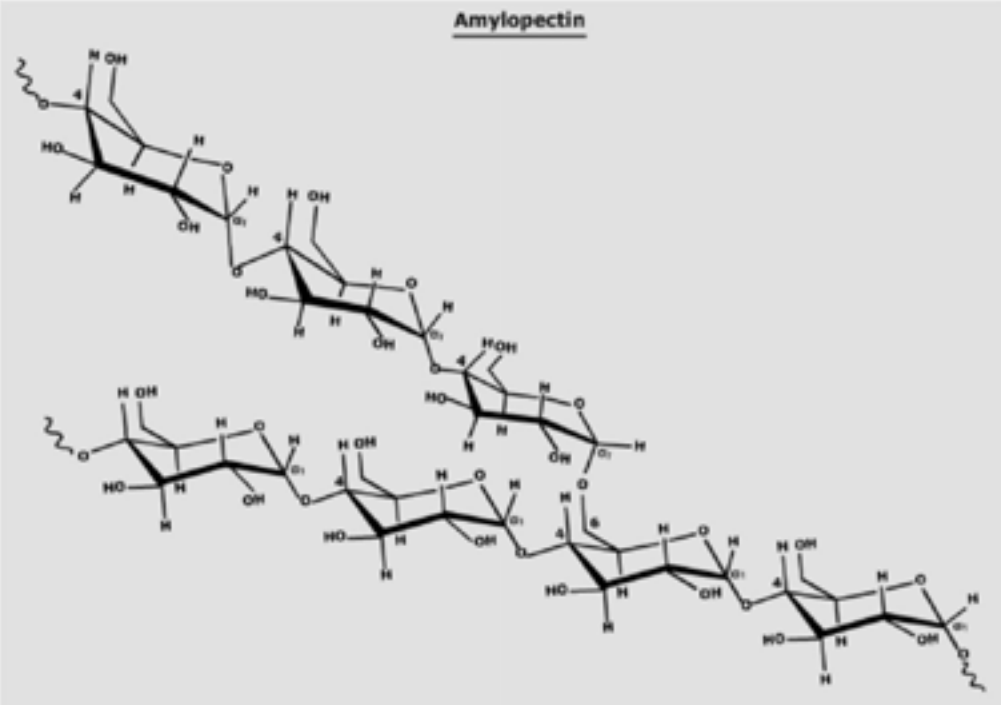


Fig-12.2: Amylopectin

Molecular weight of amylopectin range from 10^7 to 10^8 daltons, and one molecule of amylopectin may contain 50000 to 500000 molecules of D-glucose. Amylopectin gives a purple colour with iodine.

12.2.3. Gelatinization of starch

Starch gelatinization is a process that breaks down the intermolecular bonds of starch molecules in the presence of water and heat, allowing the hydrogen bonding sites (the hydroxyl hydrogen and oxygen) to engage more water. This irreversibly dissolves the starch granule. Penetration of water increases randomness in the general granule structure and decreases the number and size of crystalline regions. Crystalline regions do not allow water entry. Heat causes such regions to become diffuse, so that the chains begin to separate into an amorphous form. Under the microscope in polarized light starch loses its birefringence. Gelatinization is influenced by a number of factors. The gelatinization temperature and the length of heating, plant type (wheat and corn starch show different behaviour patterns) and the amount of water present, pH, size of starch granule. Some type of unmodified native starches start swelling at 55 °C, other types at 85 °C.

12.2.4. Retrogradation of starch

In dilute solutions, starch molecules will precipitate, with the insoluble material being difficult to redissolve by heating. The process of dissolved starch becoming less soluble is called retrogradation. Retrogradation of cooked starch involves amylose and amylopectin, with amylose undergoing retrogradation at a much more rapid rate than does amylopectin. The rate of retrogradation depends on several variables, including molecular ratio of amylose to amylopectin;

botanical source of starch; temperature; starch concentration; salts; surfactants. Bread staling is due to starch retrogradation. Staling is due to the gradual transition of amorphous starch to a partially crystalline, retrograded state.

12.2.5. Modified Starches

The behaviour of pastes of the common native starches when subjected to the effects of heat and shear used in modern food technology is often unsatisfactory. Consequently modified starches and starch derivatives with more sophisticated stability characteristics have been developed. In this section, the characteristic properties and uses of some of these starches are out-lined.

Modified starches include

(i) Acid modified starches
(ii) Pre-gelatinized starches
(iii) Cross-linked starches
(iv) Esters & ethers of starch
(v) Starch phosphates
(vi) Hydroxyalkyl substituted starch

12.2.5.1. Acid modified starch

Acid modified or thin boiling starches are prepared by heating starch granules with diluted hydrochloric acid at temperature below that of gelatinization. The resultant superficially uncharged granules fragment appear to swell less during gelatinization, with a consequent reduced volume and lower maximum hot paste viscosity. The solubility in hot water is increased, the extent depending on the degree of acid treatment.

Acid-degraded starch, particularly the non-waxy cereal type, is widely used in the manufacture of fruit gums on account of the strength and clarity of the resultant gel which is much improved in comparision to an unmodified, thick-boiling starch. The viscosity of the gel prepared from acid-modified starch is much lower than that prepared from the corresponding concentration of the unmodified starch. As a result hot gel can be easily poured into moulds. The acid treatment causes an increase in the resultant gel strength, probably because of the preferential degradation of amylopectin. The gel clarity is also improved.

12.2.5.2 Pre-gelatinized starches

For many food uses, a water holding or thickening agent is required to function without the application of heat. For this purpose a pre-gelatinized starch is often used. Pre-gelatinized starch is prepared by destroying the granular structure on cooking, which simultaneously causes a considerable reduction in paste viscosity. The cooked paste is then dried on rollers or with a spray-drier. The powdered product will easily rehydrate in cold water but, the resultant dispersions are

not equivalent to freshly prepared paste. This is due to the starch degradation which has taken place.

The largest use of pre-gelatinized starch is in the instant puddings – a packaged powder which only needs to be mixed with cold milk and allowed to stand for a few minutes, producing a simple pudding. The powders are mixture of pre-gelatinized starch with sugar and flavourings, together with salts which produce sufficient viscosity increase in the milk to keep the starch suspended until hydration can take place. Another widespread use is in frozen fruit-pie fillings where a pre-gelatinized starch keeps the fruit suspended and helps retain the flavour without the need for heating.

12.2.5.3. Cross-linked and other derivatized starches

A great number of esters and ethers of starch, with an infinite range of physicochemical properties. (particularly with regard to the heat stability), can be prepared. But use of such derivatives in food is restricted and only a few of these are important in the food industry. Starch phosphates, which have a analogues in the amylopectin fraction of root and tuber starches, are examples of starch derivatives which are suitable as food additives. The introduction of free-acid groups in starch phosphates both increases and stabilizes the paste viscosity by the

- Negatively charged phosphate groups expand the molecule in solution
- Coulombic repulsion
- Prevent the formation of aggregates

Because of their high viscosity and paste clarity, starch phosphates are put to extensive use in foods as thickness and texturizing agents. Their resistance to molecular aggregation is of importance in the formulation of frozen foods. The swelling and ultimate breakdown of starch granules during cooking can be controlled by introducing a suitable number of cross-linkages between the molecules.

Cross linked phosphate esters may be prepared commercially by esterification with trimetaphosphate. The extent of cross linking is measured by the change in the pasting properties of the derivatives. Starch with low level of phosphate cross linking is also used in textural modification of food e.g. Cross bonded phosphate starches are used as thickners in salad cream and fruit-pie fillings. The introduction of hydroxyalkyl substituent increases the solubility of starch and prevents molecular aggregation e.g. hydroxypropyl starch. Hydroxyalkyl starches gelatinize at lower temperature than the parent starch and paste show little tendency to form gels.

12.3. Cellulose

Cellulose is the most abundant polysaccharide in nature, since one-third of all higher plants consists of this biopolymer which functions as the main structural material. It is a linear polymer of D-glucose units linked (1 à 4) in the β – configuration.

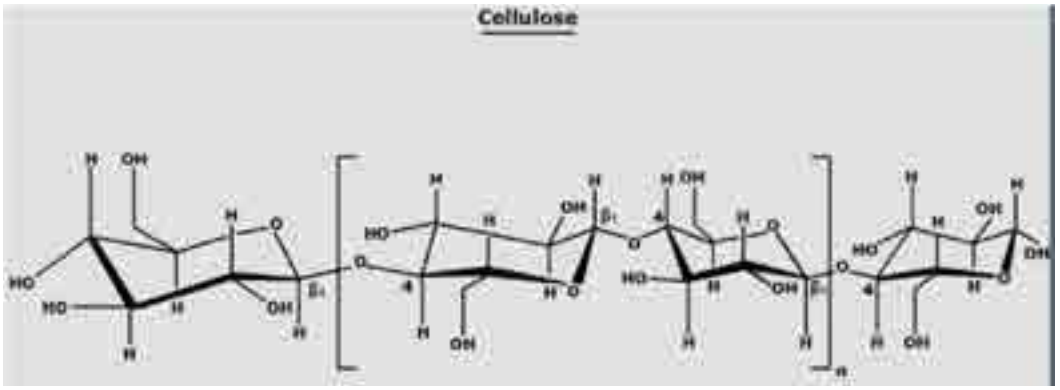


Fig-12.3: Cellulose

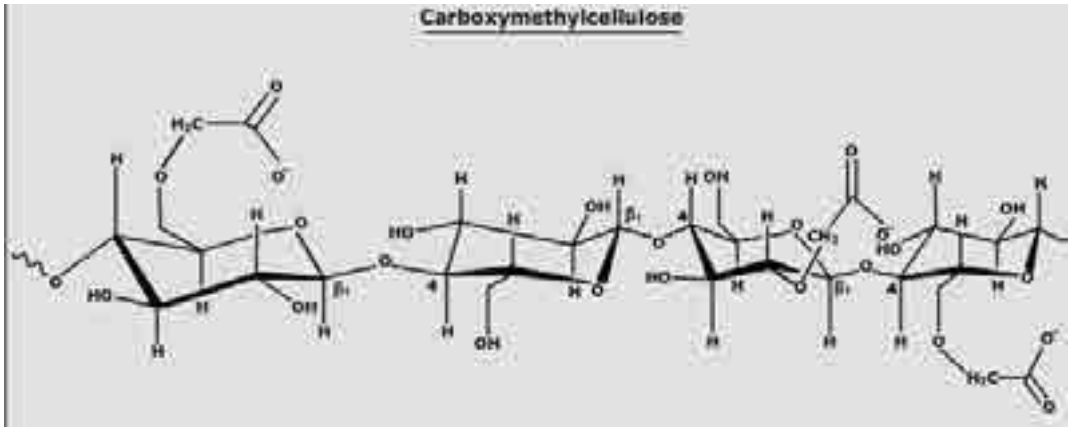


Fig-12.4: Carboxymethylcellulose

The cellulose chains are unbranched and may contain as many as 5000 glucose units. Because of the β- linkage, the glucose units in the chain alternate, and the molecule is effectively a rigid and straight chain. As a result, cellulose molecules can readily align themselves side-by-side in an arrangement which is stabilized by intermolecular hydrogen bonding and form crystalline regions. Intermolecular bonding is so strong that cellulose is insoluble in water, and even in strong sodium hydroxide solution. The cellulose is responsible for the form and gross texture in all foodstuffs prepared from plants. Being insoluble, it is little affected by any cooking process and does not disperse. On ingestion, it is unaffected by enzymes in the digestive tract and does not hydrate.

12.4. Glycogen

Glycogen the reserve carbohydrate is a polysaccharide found in the animal body. It is found mainly in the muscles (0.5 to 1 percent) and liver (3 to 7 percent).Glycogen resembles starch in its chemical properties. It is formed by the condensation of a large number (5000-10000) of glucose molecules. It is a branched chain polysaccharide, resembling amylopectin. The chain length varies from 8 to 12 glucose units. The molecular weight of glycogen from different sources range from 10^5 to 10^8 daltons.

12.5. Hemicellulose

Hemicelluloses are present in many plant tissues. They are structural components of the cell wall. They are waterinsoluble, non-starchy polysaccharides. They are heteropolysaccharides. Monosaccharide units present in hemicelluloses are xylose, arabinose, galactose, glucose, glucuronic acid. Hemicelluloses are nonfibrous while celluloses are fibrous. They are more soluble in alkali and more readily hydrolyzed by dilute acids than celluloses.

12.6. Pectin

The pectins or pectic substances are found universally in the primary cell walls & intercellular layers in plants. They are most abundant in young tissue. They are characteristic constituent of fruits e.g. citrus fruits contain 30% pectin. The pectic substances are a family of very closely associated polysaccharides which are very difficult to separate. The term 'pectin' is used in relation to water-insoluble polysaccharides.

D-galacturonic acid is the principal constituent which is esterified as methyl ester and possess considerable gelling power. Other constituents include D-galactose, L-arabinose, D-xylose, L-rhamnose and L-fucose. Three types of homopolysaccharides are also present – D-galacturonan, D-galactan, and L-arabinan. Typical heteropolysaccharides associated with pectic substance include the soyabean L-arabino-D-galactan. Preparations in which more than half of the carboxyl groups are in the methyl ester form are classified as high-methoxyl pectins, the remainder of the carboxyl groups will be present as a mixture of free acid and salt forms. Preparations in which less than half of the carboxyl groups are in the methyl ester form are called as low-methoxyl pectins. Pectin is widely used in marmalade and jelly preparation. High-methoxyl pectin solutions gel sufficient acid and sugar are present. Low-methoxyl pectin solutions gel only in the presence of calcium ions, which provide cross bridges.

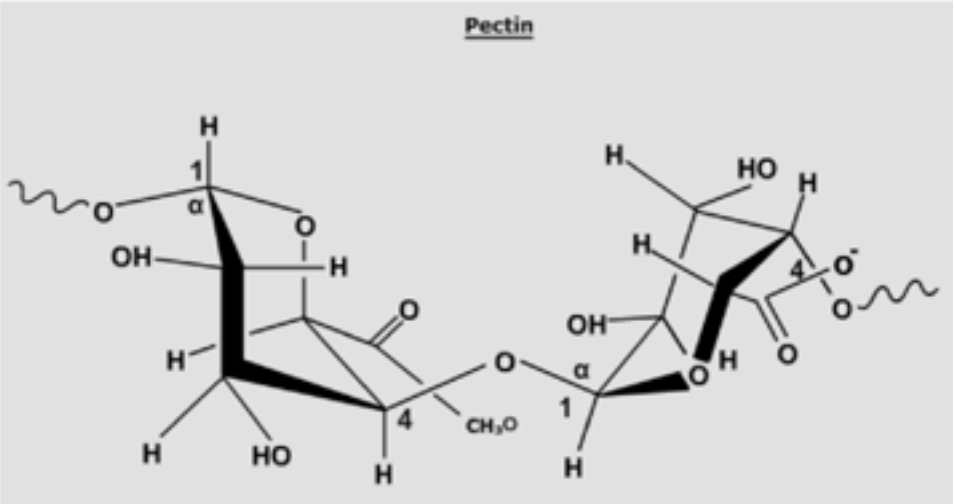


Fig-12.5: Pectin

12.7. Agar

Agar is obtained from the family of red seaweeds (*Rhodophyceae*). Example of some species are *Gelidium cartilagineum* and *Gracilaria confervoides*. Agar consists of a mixture of agarose and agaropectin. Agarose is a linear polymer. The main components of chain are β-D-galactopyranose and 3,6-anhydro-α-L-

galactopyranose, which alternate through 1, 4 and 1, 3 linkages. The chains are esterified to a low extent with sulphuric acid. Agaropectin fraction has a high sulphate esterification degree as compared to agarose fraction.

Agarose is the main gelling component of the agar.

Agar is insoluble in cold water. It dissolves to give random coils in boiling water. It forms heat resistant gels. Agar has a major use in preparation of microbiological media. Agar is added to frozen desserts and ice cream as stabilizer.

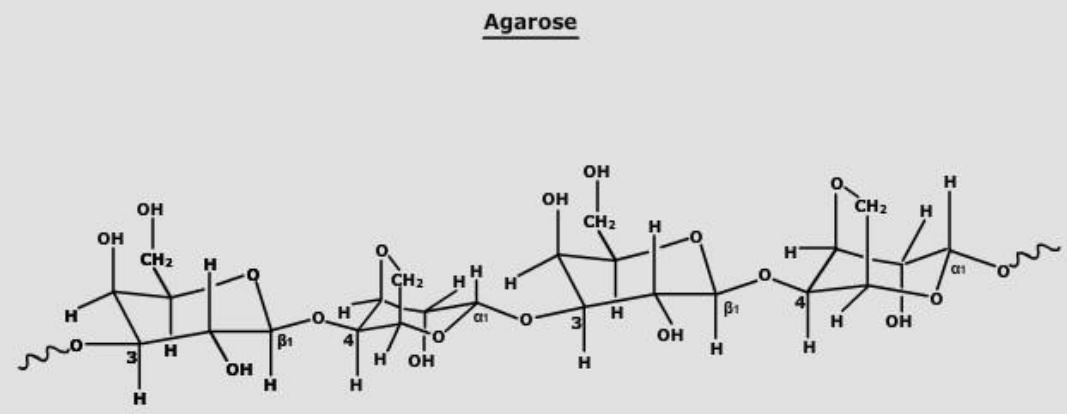


Fig-12.6: Agarose

12.8. Alginates

Alginates or Alginic acid is the most common algal polysaccharide, found in brown seaweeds (*Laminaria* spp.). This linear polysaccharide is composed of β-D-mannuronic acid and α-L-guluronic acid, both linked through the (1→4) positions. These monomer units do not occur randomly but are present in relatively long sequence of each type. It is commonly used as a gelling and stabilizing agent to improve the texture of products such as ice-cream, pie filling and icings. It forms irreversible gels in cold water in presence of calcium ions. It prevents formation of larger ice crystals in ice creams during storage.

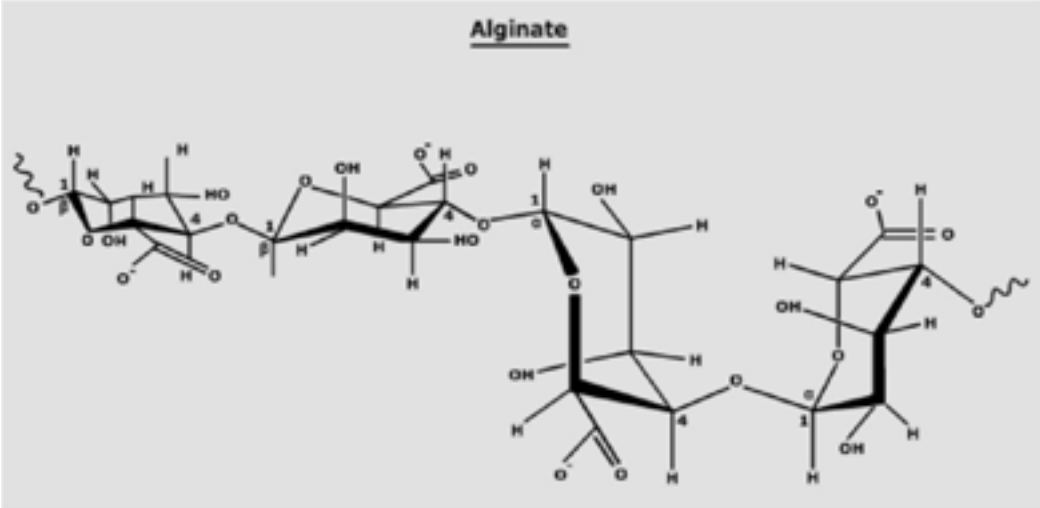


Fig-12.7: Alginate

12.9. Carrageenan

The term carrageenan covers a range of sulphated galactans which are linked alternatively by (1→3) and (1→4) glycosidic bonds. The carrageenans can be fractionated into six types which vary depending on the degree and manner of sulphation and the presence or absence of 3,6 – anhydro galactose units. These are lambda, kappa, iota, mu, nu and theta.

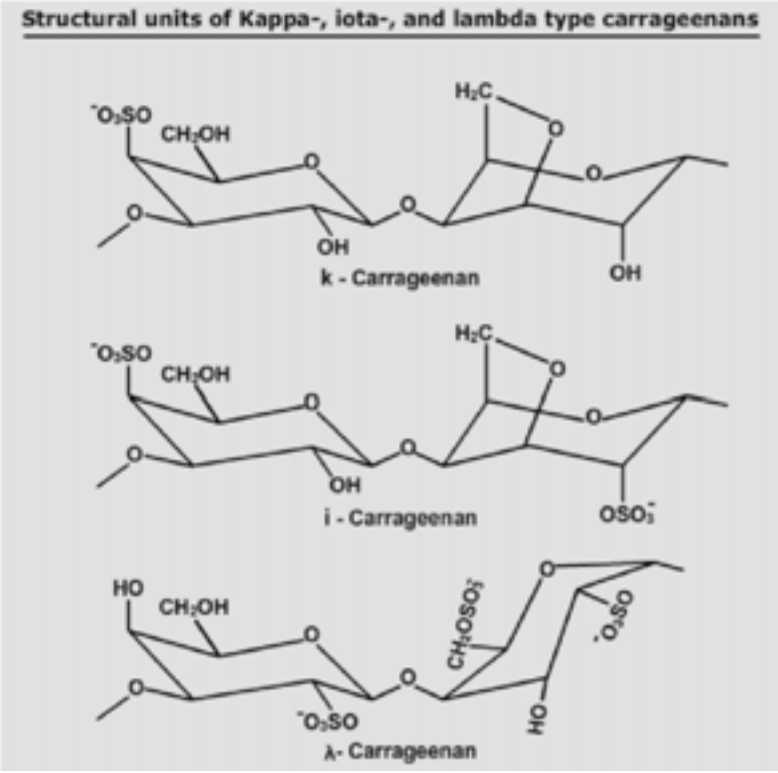


Fig-12.8: Structural units of kappa-, iota, and lambda type carrageenans

The various fractions do not occur together. The most important forms are lambda, kappa and iota. The polysaccharides have high molecular weight in the range of 1,00,000 to 10,00,000. They are regarded as being non-absorbable in the digestive tract of man.

The solubility properties of these polysaccharides depends on the

(i) Proportion of sulphate groups

(ii) Cations associated with them

(iii) Proportion of 3,6-anhydrogalactose residues (relatively hydrophobic)

Lambda fraction is easily soluble in water because of the high proportion of sulphate groups and the absence of anhydrogalactose, and is unaffected by the nature of cations present. Kappa fraction contains a lower proportion of sulphate groups and some anhydrogalactose units, and as a result is only soluble in water in the form of sodium salt. Other cations (K^+ and Ca^{2+}) only allow swelling in cold water, and heating to 60^o C is necessary to ensure solubilization. Iota fraction has an intermediate structure and properties.

Because of the presence of the strongly charged anionic sulphate group, the carrageenans as a group are able to form a complex not only with cationic materials but also with amphoteric substance such as proteins. This unique property of carrageenan extracts can be utilized is a stabilizer for condensed milk.

12.10. Gums

Gums may be formed spontaneously; or at the site of injury to the plant. They are exuded as viscous fluids which become dehydrated to give hard, clear nodules consisting mainly of polysaccharides. These are known as exudate gums. Many such gums from tropical countries find uses in the food industry as thickening agents or emulsion stabilizers. e.g. gum arabic, gum tragacanth, gum ghatti, gum karaya etc. These polysaccharides all possess complex highly-branched structure with D-glucuronic and/or D-galacturonic acids, together with two or more neutral sugars. The acidic residues are found naturally as salts and some of the sugars are esterified with acetic acid.

As a group, the gums are probably the most complex of all natural polymers and structural investigations are very difficult. Most likely, a gum is a group of closely related molecular species in which varying side chains are attached to a main backbone. Galactomannan gums such as locust bean gum and guar gum come from seeds produced by leguminous plants of *Cyamopsis* and *Ceratonia* genera. Guar gum is obtained from the ground endosperm of the leguminous plant *Cyamopsis tetragonoloba.* Guar gum consists of a linear chain of β-Dmannose units joined with 1, 4 linkages. Every second residue has a side chain, a galactose unit that is bound to the main chain by a α-1, 6 linkage. Guar gum is nongelling, and is used as a viscosity builder, stabilizer, and water binder. Guar gum is used in ice cream, desserts, salad dressings, bakery products, sauces, soups. Locust bean gum is present in the endosperm of seeds obtained from the evergreen tree, *Ceratonia siliqua*. Locust bean gum is made up of mannose and

galactose. The ratio of mannose to glucose is 4. It is insoluble in cold water. It is compatible with other gums. It readily forms gel when combined with xanthan gum. Functions of locust bean gum include thickening, stabilization of emulsion and inhibition of syneresis. It is used in sauces, beverages, cheese, ice cream.

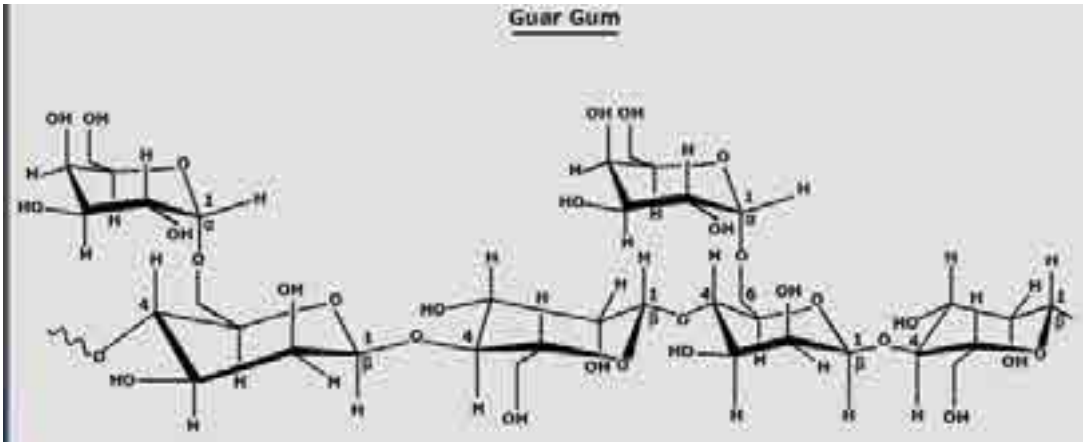


Fig-12.9: Guar Gum

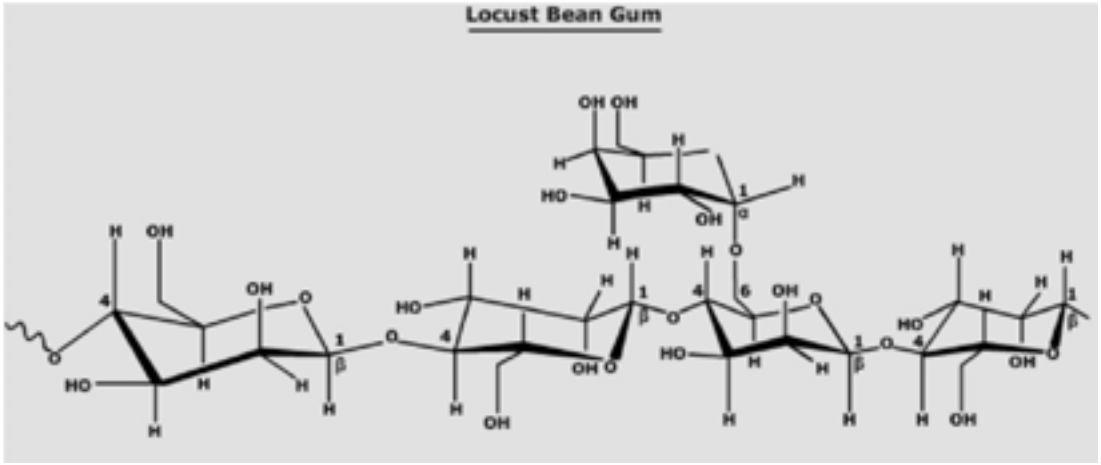


Fig-12.10: Locust Bean Gum

Enzymatic degradation of polysaccharides – Starch Production of dextrins and maltodextrins

13.1. INTRODUCTION

Starch is the commonest storage carbohydrate in plants. It is used by the plants themselves, by microbes and by higher organisms so there is a great diversity of enzymes able to catalyse its hydrolysis. Starch from all plant sources occurs in the form of granules which differ markedly in size and physical characteristics from species to species. Chemical differences are less marked. The major difference is the ratio of amylose to amylopectin; e.g. corn starch from waxy maize contains only 2% amylose but that from amylomaize is about 80% amylose. Acid hydrolysis of starch had widespread use in the past. It is now largely replaced by enzymatic processes. Acid hydrolysis requires the use of corrosion resistant materials which gives rise to high colour, salt and ash content (after neutralisation), needs more energy for heating and is relatively difficult to control.

13.2 AMYLASES

Enzymes involved in degradation of starch belong to hydrolases (Glycosidases). Amylases are the most important starch degrading enzymes. They hydrolyze the starch to oligosaccharides and simple sugars. Of the two components of starch, amylopectin presents the great challenge to hydrolytic enzyme systems. This is due to the residues involved in α-1,6-glycosidic branch points which constitute about 4 - 6% of the glucose present. Most hydrolytic enzymes are specific for α-1,4-glucosidic links yet the α-1,6-glucosidic links must also be cleaved for complete hydrolysis of amylopectin to glucose. The following are the most important enzymes.

13.2.1. α-amylase: α-amylase is an endoenzyme. It hydrolyzes the α-1,4 glycosidic bonds randomly along the chain. Amylopectin is hydrolyzed to oligosaccharides that contain two to six glucose units. The branch points are over jumped. A mixture of amylose and amylopectin is hydrolyzed into a mixture of dextrins, maltose and glucose. Amylose is completely hydrolyzed to maltose. Calcium ions are required for its activation. α-amylase cleaves both amylose and amylopectin molecules producing oligosaccharides. Oligosaccharides of 6-7 glucose units are released from amylose. α-amylase activity leads to a rapid decrease in viscosity of starch solution.

Enzymatic hydrolysis is increased by the gelatinization of starch. α-amylase hydrolyzes the α-1,4-bonds of amylose and amylopectin in a random manner, liberating small units with free non-reducing end groups. Low molecular weight dextrins are formed.

13.2.2. β-amylase: β-amylase also hydrolyzes the α-1,4-bonds of amylose and amylopectin, removing maltose units from the non-reducing end of starch in an orderly fashion. The α-amylase and β-amylase do not cleave the α-1,6-linkages in amylopectin.

13.2.3. Glucoamylase: Glucoamylase is used in combination with an α-amylase to produce D-glucose syrups and crystalline D-glucose. The enzyme acts upon fully gelatinized starch sequentially releasing single D-glucosyl units from the nonreducing ends of amylose and amylopectin molecules.

13.2.4. Pullulanase: Pullulanase hydrolyzes α-1,6 glucosidic bonds in polysaccharides, e.g. in amylopectin, glycogen, and pullulan. Linear amylose fragments are formed from amylopectin.

13.3. PRODUCTION OF DEXTRINS AND MALTODEXTRINS

Dextrins: Dextrins are a group of low-molecular-weight carbohydrates produced by the hydrolysis of starch.

Dextrins are mixtures of polymers of D-glucose units linked by α-(1→4) or α-(1→6) glycosidic bonds. They are less complex than starch.

Dextrins can be produced from starch using enzymes like amylases or by applying dry heat under acidic conditions. The latter process is used industrially, and also occurs on the surface of bread during the baking process, contributing to flavour, colour, and crispness. Dextrins are produced by heating starch with hydrochloric acid or phosphoric acid at levels of 0.15 to 0.17 % to attain desired degree of polymerization. Dextrins produced by heat are also known as pyrodextrins. During the hydrolysis of starch to maltose by amylases, starch is broken down to dextrins of decreasing molecular weight before all the starch is converted into maltose.

Dextrins have adhesive and film forming properties. They are used as binders, fillers, encapsulating agents and carriers of flavour. Dextrins are used as a crispness enhancer for food processing, in food batters, coatings, and glazes.

Maltodextrins: Maltodextrins are polysaccharides that are used as a food additive. They are produced from starch by partial hydrolysis and are usually found as a creamy-white hygroscopic spray dried powder. Maltodextrins consist of D-glucose units connected in chains of variable length. The glucose units are primarily linked with α(1→4) glycosidic bonds.

Maltodextrins are typically composed of a mixture of chains that vary from three to seventeen glucose units long. Maltodextrins are classified by DE (dextrose equivalent) and have a DE between 3 to 20. The higher the DE value, the shorter the glucose chains, the higher the sweetness, the higher the solubility and the lower heat resistant. Above DE 20 it is glucose syrup. DE of a product of hydrolysis is its reducing power as a percentage of the reducing power of pure dextrose. Maltodextrins of lowest DE are non hygroscopic, while those of highest DE tend to absorb moisture.

Maltodextrins are easily digestible, being absorbed as rapidly as glucose, and might be either moderately sweet or almost flavourless. Maltodextrins provide bulk to food systems. They are commonly used for the production of sodas and candy. It can also be found as an ingredient in a variety of other processed

foods. Maltodextrins are a common adjunct to beer brewing to increase the specific gravity of the final beer product.

Hydrolases, lipases and other important enzymes in food

15.1. INTRODUCTION

Enzymes are biological catalysts which are proteinaceous in nature having a specific catalytic site called active centre.

In some cases enzymes contain a nonprotein part called "**cofactor".** The protein portion is designated the **"apoenzyme"**. Without its cofactor it is catalytically inactive. The fully intact enzyme is sometimes referred to as the **holoenzyme**. The relation expressed in word equation is

Cofactor + apoenzyme ® Holoenzyme (or enzyme)

Cofactor can be a simple divalent metallic ion (e.g. Mg^{2+}, Ca^{2+}, Zn^{2+}, Co^{2+}, or Mn^{2+}).

Cofactor can be a non protein organic compound. If the cofactor is firmly bound to the apoenzyme it is known as prosthetic group. If the cofactor is loosely bound to the apoenzyme it is known as coenzyme. Cofactors are generally stable to heat, whereas most enzyme proteins lose activity on heating.

Proenzyme or zymogen : Some of enzymes are produced in the inactive form which are called proenzymes or zymogens which can be converted into active form. Proenzyme – Pepsinogen ; Enzyme - Pepsin

Proenzyme – Trypsinogen ; Enzyme - Trypsin

15.2. PROPERTIES OF ENZYMES

1. Enzyme molecules not only are largely protein in nature but are very often much, much larger than the molecules of the chemical or chemicals whose reactions they catalyze.

Since the enzyme molecule is usually much larger than its substrate, it is believed that the latter can occupy a limited area on the enzyme surface. This area to which the substrate becomes bound is known as the active site or active centre of the enzyme and must bear a specific complementary relationship to the structure of the substrate (s) which allows an almost precise fit between the. The active site is made up of : a) a binding site , and b) a catalytic site. Only a few of the amino acids in the peptide chain take part in the catalytic mechanism, while others, which presumably adjoin or overlap the catalytic site,

determining the specificity of the enzyme. As might be expected, the active site usually includes amino acids, such as serine, histidine and cysteine, which have reactive side-chain grouping.

2. Specificity of enzymes for their substrates is one of the most striking proposals of the enzyme molecule. Although this phenomenon is exhibited by inorganic catalysts, the enzymes are far more selective and discriminating in their specificity requirements. Enzyme specificity depends on the particular atomic structure and configuration of both the substrate and the enzyme.

3. The rate of enzyme-catalysed reactions are extraordinarily more rapid than the same or similar reactions subject to nonenzymic catalysis.

4. Enzymes promote reactions under relatively mild temperatures.

5. Enzymes promote reactions at nearly neutral pHs.

6. In contrast to inorganic catalysts enzymes are synthesized under the direction of genes and consequently regulated by factors influencing those genes.

7. One of the distinctive feature observed by enzyme-catalyzed reactions but not usually observed in nonenzymatic reactions is saturation of enzyme with substrate.

15.3. NOMENCLATURE AND CLASSIFICATION OF ENZYMES

Enzymes are classified by the Commission on Enzymes of the International Union of Biochemistry. The basis of classification is the division of enzymes into six major classes and sets of subclasses, according to the type of reaction catalyzed. Each enzyme can be described in three ways – By a trivial name: usually short and appropriate for everyday use, a

By a systematic name: which identifies the reaction it catalyzes, and

By a number of the Enzyme Commission (EC): which is used where accurate & unambiguous identification of an enzyme is required, as in international research journals, abstracts and indexes.

The six major classes of enzymes are:

1. **Oxido-reductases** are involved in oxidation-reduction reactions.

They oxidize or reduce substrates by transfer of hydrogen or electrons or

by oxygen. An example is, catalase (EC 1.11.1.6).

2. **Transferases** are involved in transfer of functional group. They remove groups from substrates and transfer them to acceptor molecules. An example is, glucokinase

(EC 2.7.1.2).

3. **Hydrolases** are involved in hydrolysis reactions. These enzymes catalyze hydrolysis of ester, thioester, peptide, glycosyl, acid anhydride by the addition of water. For a substrate XY, the reaction can be represented as follows:

XY + HOH ------- HX + YOH

An example is alkaline phosphatase (EC 3.1.3.1).

4. **Lyases** are enzymes that catalyze the cleavage of C-C, C-O, C-N and other groups by elimination (not by hydrolysis), leaving double bonds, or conversely adding groups to double bonds. An example is fumarate hydratase (EC 4.2.1.2).

5. **Isomerases** are involved in the catalysis of isomerizations within one molecule. An example is mutase (EC 5.4.2.1).

6. **Ligases** are involved in the formation of bonds with ATP cleavage. They are involved in the biosynthesis of a compound with the simultaneous hydrolysis of a pyrophosphate bond in ATP. Alkaline phosphatase

Trivial name : alkaline phosphatase

Systematic name : orthophosphoric monoester phosphohydrolase

Reaction catalysed An orthophosphoric monoestser + H_2O « An alcohol + H_3PO_4

Classification number : EC 3.1.3.1, where EC stands for Enzyme Commission

The first digit (3) for the class name (hydrolases)

The second digit (1) for the subclass (acting on ester bonds)

The third digit (3) for the sub-subclass (phosphoric monoester)

The fourth digit (1) designates alkaline phosphatase

Lipase

Recommended name : lipase

Systematic name : glycerol ester hydrolase

Reaction catalysed A triglyceride + H_2O « A diglyceride + a fatty acid

Classification number : EC 3.1.1.3, where EC stands for Enzyme Commission

The first digit (3) for the class name (hydrolases)

The second digit (1) for the subclass (acting on ester bonds)
The third digit (1) for the sub-subclass (carboxylic ester)

The fourth digit (3) designates lipase

15.4. HYDROLASES

Most of the enzymes used in the food industry belong to the class of hydrolase enzymes. Some of them are described below.

15.4.1. Amylases α-amylase hydrolyzes the α-1,4-bonds of amylose and amylopectin in a random manner, liberating small units with free non-reducing end groups. Low molecular weight dextrins are formed. β-amylase also hydrolyzes the α1,4-bonds of amylose and amylopectin, removing maltose units from the non-reducing end of starch in an orderly fashion. The α-amylase and β-amylase do not cleave the α-1,6-linkages in amylopectin.

The use of amylases is important in bread making and in the manufacture of corn syrups. In bread making, during fermentation period, α-amylase present in flour catalyzes the dextrinization of the damaged starch granules. These dextrins are further hydrolyzed by β-amylase and converted to maltose, which provides the fermentable sugar for the yeast cells. During baking process, as the oven temperature rises the activity of α-amylase is destroyed. The application of amylases produce a bread with a softer crumb, deeper crust colour, greater volume, and improved grain and texture.

The conversion of starch into sweet syrups e.g. corn syrup is a combination of acid and enzymatic hydrolysis. A fungal amylase preparation consisting of α-, β- and amylo-1,6-glucosidase is used to

produce a well flavoured, low viscous syrup consisting of dextrose, maltose, and a small amount of dextrin.

15.4.2. β-D-Fructofuranosidase (Invertase)

This enzyme plays an important role in the confectionary industry. It is involved in hydrolysis of sucrose. The products of hydrolysis, invert sugar consist of equimolar amounts of glucose and fructose and have a much sweeter taste than the original sucrose. **15.4.3. Pectinolytic Enzymes**

Pectic enzymes act on pectic substances. They include pectin methylesterase, polygalacturonase, pectate lyases.

Pectin methylesterase hydrolyzes the methyl ester bond of pectin to give pectic acid and methanol. Pectic acid flocculates in the presence of Ca^{2+} ions.

Polygalacturonase hydrolyzes the α-1,4-glycosidic bond between the anhydrogalacturonic acid units.

Pectinolytic enzymes are used for the clarification of fruit and vegetable juices.

15.4.4. Glucoamylase

Glucoamylase cleaves β-D-glucose units from the non-reducing end of an 1,4-α-D-glucan. The α-1,6-branching bond present in amylo-pectin is cleaved at a rate about 30 times slower than the α-1,4-linkages occurring in straight chains. The enzyme preparation is produced from bacterial and fungal cultures. The removal of transglucosidase enzymes which catalyze, for example, the transfer of glucose to maltose, thus lowering the yield of glucose in the starch saccharification process, is important in the production of glucoamylase. In a purely enzymatic process, the swelling and gelatinization and liquefaction of starch can occur in a single step using heatstable bacterial α-amylase. The action of amylases yields starch syrup which is a mixture of glucose, maltose and dextrins.

15.4.5. β-D-Galactosidase (Lactase) β-D-Galactosidase catalyzes the hydrolysis of lactose into glucose and galactose. Enzyme preparations from fungi (*Aspergillus niger*) or from yeast are used in the dairy industry to hydrolyze lactose. Immobilized enzymes are applied to produce milk suitable for people suffering from lactose intolerance.

15.4.6. Proteases

The reaction catalyzed by proteases (proteolytic enzymes) is the hydrolysis of peptide bonds of proteins. Most of the proteolytic enzymes used in the food industry endopeptidases. These enzymes are isolated from animal organs, higher plants or microorganisms. They are important in many industrial food processing procedures. Examples of their utilization are as follows. In the dairy industry, in cheese manufacture, the formation 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. Proteinases from *Mucor miehei, Mucor pusillus* and *Endothia parasitica* are a suitable replacement for rennin. 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.

Haze is a result of the combination of polypeptide and tannin molecules in beer giving rise to easily observed particles. Proteolytic enzymes (papain, pepsin, ficin, bromelain and microbial proteases) prevent this type of haze by reducing the polypeptide size. Papain, ficin and bromelain are sulphydryl proteases. These enzymes catalyze the hydrolysis of peptide, ester and amide bonds.

Proteases are added to wheat flour in the production of some bakery products to modify rheological properties of dough and, thus, the firmness of the endproduct. During such dough treatment, the hard wheat gluten is partially hydrolyzed to a soft-type gluten. Proteases are used for tenderization of meat. The enzymes hydrolyze one or more of the muscle tissue components. The enzymes are trypsin, papain, bromelain, ficin, etc.

15.4.7. Lipases

Lipases play a major role in cheese manufacture. Lipases hydrolyze ester linkage in glycerides. Lipase from microbial sources is utilized in cheese ripening for development of aromas. Lipases are responsible for hydrolytic rancidity in dairy products. Staling of bakery products is retarded by lipase, presumably through the release of mono- and diacylglycerols. The defatting of bones, which has to be carried out under mild conditions in the production of gelatin, is facilitated by using lipase-catalyzed hydrolysis.

15.5. Oxidoreductases are involved in oxidation-reduction reactions. They oxidize or reduce substrates by transfer of hydrogen or electrons or by oxygen.

15.5.1. Glucose Oxidase

Glucose oxidase is used to remove traces of glucose and oxygen from food products such as beer, wine, fruit juices, mayonnaise etc. It can be used as an analytical reagent for the specific determination of glucose. Glucose oxidase oxidizes glucose to gluconic acid in presence of oxygen and hydrogen peroxide. Hydrogen peroxide decomposes into water and oxygen in the presence of catalase.The enzyme is produced by fungi such as *Aspergillus niger* and *Penicillium notatum*.

15.5.2. Catalase

Catalase catalyzes the decomposition of hydrogen peroxide into water and molecular oxygen. In plants, catalase has the ability to dispose of the excess H_2O_2 produced in oxidative metabolism and to use H_2O_2 in oxidation of phenols, alcohols and other hydrogen donors. Catalase is used in combination with glucose oxidase.

15.5.3. Ascorbic Acid Oxidase

Ascorbic acid oxidase catalyzes the following reaction.

L-Ascorbic acid + ½ O_2 ---------- dehydroascorbic acid + H_2O

The reaction is significant in fruits and vegetables. It is responsible for the initiation of browning reaction, and for the eventual loss of all vitamin C activity.

15.5.4. Lipoxygenase

Lipoxygenase is utilized in the bleaching of flour and the improvement of the rheological properties of dough.

15.5.5. Peroxidase

Peroxidase catalyzes the following reaction

$H_2O_2 + AH_2$ --------- $2H_2O + A$
AH_2 is an oxidizable substrate.

The common plant peroxidases are iron containing peroxidases. Peroxidases of animal tissue and milk (lactoperoxidase) are flavoprotein peroxidases. The peroxidase test is used as indicator of satisfactory blanching of fruits and vegetables. **15.5.6. Phenolases**

Phenolases are involved in enzymatic browning. They are also known as polyphenoloxidases or polyphenolases. These enzymes have the ability to oxidize phenolic compounds to o-quinones. High levels of these enzymes are present in potatoes, apples, peaches, bananas, tea leaves, coffee beans etc. The action of phenolases is undesirable when it leads to browning in bruised and broken plant tissue but it is desirable in the processing of tea and coffee.

Vitamins, Amino Acids, Minerals

17.1. INTRODUCTION

A food additive is a substance (or a mixture of substances) which is added to food and is involved in production, processing, packaging and/or storage of foods without being a major ingredient. Additives or their degradation products generally remain in food, but in some cases they may be removed during processing.

Food additives are defined in various ways:

17.2. DEFINITION

Food additives may be defined as chemical substances which are deliberately added to foods, in known and regulated quantities, for the purpose of assisting in the processing of foods, preservation of foods or in improving the flavor and texture or appearance of foods.

17.2.1. Definition according to PFA

As per PFA Act food additive is defined as any substance not normally used as a typical ingredient of foods whether or not it has nutritional value; the intentional addition of which to food for technological Including organoleptic purpose in the manufacturing, processing, preparation; treatment, packing, packaging, transport or holding of food results in it or its ingredients becoming a component or otherwise affecting characteristics of such foods.

17.3. FUNCTIONS OF FOOD ADDITIVES

Different food additives perform several useful functions in the interest of manufacturer and consumer of the food and food products. Some important functions are listed below:

1. Enhances the shelf life of food.
2. Improves and maintains the nutritive value of food.
3. Reduces the wastage and improves yield of the product.
4. Facilitates the processing/preparation of food.
5. Improves colour and appearance of food.
6. Improves body and texture of food.
7. Improves aroma and taste of food.
8. Enhance the consumer's acceptability of the food.

17.4. CLASSIFICATION OF FOOD ADDITIVES

They are classified into

two ways

(A) Intentional

FoodAdditives

These are those substances added to food intentionally to improve product quality and sensory properties. These are generally added to foods selectively in carefully controlled conditions during processing and in small permissible amounts necessary to achieve the desired effects e.g. Preservatives, antioxidants, emulsifying agents, stabilizers, flavorings, colourants, nutrient supplements etc.

(B) Unintentional Food Additives - contaminants

These are those additives which are not deliberately added to foods but gain entry as a result of operations inherent to production, storage, processing or marketing. They find their way in food accidentally. Some of the incidental additives are pesticides, toxic metals, anti nutrients, heavy metals etc. It may cause health hazard and may also spoil the food.

17.5. FOOD ADDITIVES ARE FURTHER CLASSIFIED BASED ON SOURCE

They are natural, synthetic and nature identical

Natural

They are derived from natural sources like animals, plants, micro-organisms etc.

Synthetic:

They are chemically synthesized in laboratory

Nature Identical:

They chemically identical to those obtained from natural sources but synthesized artificially.

17.6. VARIOUS CATEGORIES OF FOOD ADDITIVES

1. Preservatives
2. Antioxidants
3. Appearance control agents – BVO, ester gum, waxes, polishes etc
4. Coloring agents
5. Flavour enhancers
6. Emulsifiers and Stabilizers(Thickening agents)
7. Humectants – moisture control agents
8. Sugar substitutes and artificial agents
9. Nutrients supplement (vitamins, amino acids, minerals, etc.)
10. Buffers – pH control agents – acids, alkalis and salts
11. Leavening agents – yeast and chemicals
12. Propellants and gases
13. Oxidizing and reducing agents
14. Sequestering agents and chelating agents
15. Firming agents
16. Masticating substances
17. Anti-stick (release) and Anti-caking (free- flowing) agents
18. Tracers
19. Anti-freeze agents
20. Bulking agents
21. Clarifying agents
22. Bleaching & maturing agents
23. Acidulants
24. Foaming (aerating) and Antifoaming agents

17.7. CONSIDERATIONS REQUIRED IN USE OF FOOD ADDITIVES

The following criteria/guidelines are required to be taken care of before the use of any additives.

1. It must be ascertained that the real need exists for the use.
2. It does not cause any adverse physiological and harmful effects even upon regular consumption for a prolong period i.e. the food additives must be safe/ harmless.
3. It should not reduce/destroy the nutritive value of food.

4. It should confirm the agreed specifications, where possible legislation should define permissible maximum quantities of a given additive.

Any food additive should be used at minimum level necessary to produce the desired effect, additives or their degradation products generally remain in food but in some cases they may be removed during processing. The limit of addition should be established based on the following factors:

1. The estimated level of the consumption of food for which an additive is proposed.
2. Minimum level which in animal studies exhibit minimum deviation from the normal physiological behaviour.
3. An adequate margin of safety to reduce to a minimum any hazard to health in all groups of consumers.

17.8. SAFETY ASPECTS OF FOOD ADDITIVES

It is necessary to know in advance how safe the food additive is before permitting its use in food products.

ADI of Food Additives:

The ADI (Acceptable Daily Intake) is the amount that can be consumed on a daily basis for a life time without appreciable risk. Its unit is mg/kg body weight / day.

GRAS substances:

GRAS – Generally Recognized as Safe

It is a device which US FDA has adopted to give endorsement to those substances which have had many years of use and for which there is no evidence of any harmful effects.

17.9. VITAMINS

Many food products are enriched or fortified with vitamins to adjust for processing losses or to increase the nutritive value. Such enrichment is important, particularly for fruit juices, canned vegetables, flour and bread, milk, margarine and infant food formulations. Table given below provides an overview of vitamin enrichment of food. Several vitamins have some desirable additional effects. Ascorbic acid is a dough improver, but can play a role similar to tocopherol as an antioxidant. Carotenoids and riboflavin are used as coloring pigments, while niacin improves the color stability of fresh and cured and pickled meat.

Table 17.1. Examples of vitamin fortification of food products

Vitamin	Food products
B1	Cocoa powder and its products, beverages and concentrates, confectionary and other baked products
B2	Baked products, beverages
B6	Baked and pasta products
B12	Beverages, etc.
Pantothenic acid	Baked products
Folic acid	Cereals
C	Fruit drinks, desserts, dairy products, flour
A	Skim milk powder, breakfast cereals (flakes), beverage concentrates, margarine, baked products, etc.
D	Milk, milk powder, etc.
E	Various food products, e. g. Margarine

17.10. AMINO ACIDS

The biological value of a protein (g protein formed in the body/100 g food protein) is determined by the absolute content of essential amino acids, by the relative proportions of essential amino acids, by their ratios to nonessential amino acids and by factors such as digestibility and availability. Since food is not

available in sufficient quantity or quality in many parts of the world, increasing its biological value by addition of essential amino acids is gaining in importance. The best example of use of amino acid as additive is fortification of rice with L-lysine and L-threonine, supplementation of bread with L-lysine and fortification of soya and peanut protein with methionine. Synthetic amino acids are used also for chemically defined diets which can be completely absorbed and utilized for nutritional purposes in space travel, in pre-and post-operative states, and during therapy for maldigestion and malabsorption syndromes.

17.11. MINERALS

Food is usually an abundant source of minerals. Fortification is considered for iron, which is often not fully available, and for calcium, magnesium, copper and zinc. Iodization of salt is of importance in iodine deficient areas.

Iodized salt is produced as a preventive measure against goiter, a disease of the thyroid gland. It contains 5 mg⁄kg of sodium-, potassium- or calcium iodide. Nitrite salts are used for pickling and dry curing of meat. They consist of common salt and sodium nitrite (0.4–0.5%), with or without additional potassium nitrate.

17.11.1. Fortification is the practice of deliberately increasing the content of an essential micronutrient, i.e. Vitamins and Minerals (including trace elements) in a food, so as to improve the nutritional quality of the food supply and provide a public health benefit with minimal risk to health.

Food colours / pigments

20.1. INTRODUCTION

Colour is the first sensory quality by which foods are judged; food quality and flavour are closely associated with colour. Colour far outweighs flavour in the impression it makes on the consumer even when the flavour are pleasant. Colour powerfully influences the consumer's ability to identify the flavour and quality. Colour is the general name of the all sensations arising from the activity of the retina of eye. Colour is important to many foods, both that are unprocessed and manufactured. Together with flavour and texture, colour plays an important role in food acceptability. The colours of foods are result of natural pigments or of added colours. Colour compounds are a unique class considering their structural diversity and extremely complex chemical and physical properties.

20.3. IMPORTANCE OF FOOD COLOURS

As food should also be attractive to the eye, colour plays a key role in defining its quality. Colour is the first characteristic of the food that is noticed and it determines our expectation of both flavour and quality. Colorants affect the identification of flavor as well as it affects sensing the actual level of sweetness in the food.

To overcome the damage to the appearance caused by processing and to preserve product identity
To ensure colour uniformity of food products that naturally vary in colour
To intensify the colours of certain manufactured foods
To help protect flavour and light sensitive vitamins during storage by a sunscreen effect
To serve as a visual indication of quality
To give colour to certain foods such as sugar confectionery, soft drinks, sauces, ice lollies and soft drinks, this would otherwise be virtually colourless.

WHY FOOD PRODUCTS NEED TO BE COLOURED?

Absence of any adverse reaction, on regular and prolonged consumption is the main requirement in choice of a dye as food additive. It is also necessary that it should impart attractive and natural colour to food.

As food should also be attractive to the eye, colour plays a key role in defining its quality. Colour is the first characteristic of the food that is noticed and it determines our expectation of both flavour and quality. Colorants affects the identification of flavor as well as it affects the sensing the actual level of sweetness in the food.

To overcome the damage to the appearance caused by processing

To preserve the product identity

To ensure colour uniformity of food products that naturally vary in colour

To intensify the colours of certain manufactured foods

To help protect flavour and light sensitive vitamins during

storage To serve as a visual indication of quality

20.5. CLASSIFICATION OF FOOD COLOURS

Colours added to food are regulated as food additives. In foods, colouring matter means those substances that when added restores or adds the colour in foods. Synthetic colourants used commercially are also known as certified colour additives. The added colourants can be classified as:

NATURAL COLOURS: Natural colourants are those that are extracted from animals, vegetables, fruits, minerals and spices used to colour foods. e.g. carotenoids from annatto, paprika, saffron, anthocyanins, caramel, chlorophyll and turmeric. Carotenoids are used the most followed by the red pigment and brown coloured caramels.

Anthocyanins

Anthocyanins are the water soluble compounds responsible for the red to blue colour of variety of fruits and vegetables. It can be derived from various sources including grapes, redcurrants and blackcurrants, raspberries, strawberries, apples, cherries, red cabbages, bringle. They provide orange, red, blue, violet and magenta colours.

The use of anthocyanins dates back to antiquity as Romans used highly coloured berried to augment the colours of wine.

Carotenoids

Carotenoids are widely spread natural pigments in plants and animals. It is estimated that nature produces some 3.5 tonnes of carotenoids every second. Over 600 different carotenoids have been identified and many of these are present in our diet.

They provide natural yellow, orange or red colours of many food as well as being used extensively non-toxic natural or nature-identical colorants. Chemically the carotenoids are aliphatic or alicyclic members of terpene group. Eight isoprene units joined in a tail-to-tail manner at the center of the molecule. The carotenoids can be divided into hydrocarbon carotenes and their oxygenated derivatives, called xanthophylls (violaxanthin, neoxanthin etc.).

β-carotene

Beta carotene occurs in nature usually associated with a number of chemically closely related pigments and extracts have been used as food colorants for many years. It was first isolated from carrots and hence the name carotene was given to this yellow pigment. The carrot represents the most commonly known source of carotene. It also occurs in a wide variety of other fruits and vegetables including banana, jack fruit, maize, mango, papaya, pumpkin, watermelon, red pepper, spinach, peaches, apricots, oranges, broccoli, etc.

It imparts yellow-to- orange colour in foods. It is used at a concentration of 0.13% to 2%. The most important application of oil soluble form of β-carotene is for colouring butter and margarine. In water-based products like ice-cream, yoghurts, etc., water soluble nor-bixin products are used.

β -apo-8'-carotenal

Beta-apo-8'-carotenal is found in abundance in the vegetable kingdom, e.g. in the pulp and skin of citrus fruits and in various fodder plants including oranges, spinach, grass and marigold. It was first synthesized in the year 1962.

Certain specifically developed β-apo-8'-carotenal formulations products may be used in food products like cheese, imitation dairy products, pastry, whipped margarine, non-standardized salad dressings and fresh dressing.

Canthaxanthin

Canthaxanthin is a diketo carotenoid pigment with an orange-red colour. It occurs in the edible mushroom, chanterelle (*Cantharellus cinnabarinus*), in the plumage and organs of flamingoes, the scarlet ibis (*Guara rubra*), and the roseate spoonbill (*Ajaja ajaja*), and in various crustacea and fish (trout, salmon). Canthaxanthin is the principal pigment of the pink edible mushroom, *Cantharellus cinnabarina.* It is also isolated from algae, hydra and the brine shrimp. It widely occurs in water birds that feed on crustacean. Thus it is a major pigment of several flamingo species, occurring in their feathers, leg, skin, egg yolk, blood plasma a d liver. It was first synthesized chemically in the year 1964.

Canthaxanthin is used at 5 to 60 ppm levels to impart red colour to food products. It blends well with β-carotene to produce orange shades. Canthaxanthin is frequently used to enhance and standardize the colour of tomato products like juice, sauce, soup, and dehydrated powder. The other food applications include Russian and French dressings, fruit drinks, and ice cream.

Annatto

Annatto is a natural colorant derived from pericarp of annatto (*Bixa orellana L.*) seeds. Annatto is fast growing shrub which produces cluster of pods containing 10 to 50 seeds. The seeds are covered with thin pulpy, bright orange resinous coating which serves as a source of colour.
Annatto colour is generally used at a level of 0.5 to 30 ppm in food products resulting in hue ranging from light yellow to dark orange. The type of colour preparation employed and the product to be coloured also dictate the end effect.

Oil-soluble annatto was formerly used in fat-based products like butter and margarine. However now it is also used in creams, spreads, desserts, etc. Water soluble annatto was traditionally used in cheese and cheese products.

Betalain

Betalain is found in wide range of fruits, vegetables, leaves of some plants and in underground part of beet-root.

Among the different phenolic compounds that are relevant in plant foods, indigoids and indol derivatives represent the largest class. Betalain is the most noticeable group among indigoids. The betalain contain nitrogen in their ring structure and also contain glycoside residue. Betalain is defined as ‘a water soluble, indigoid pigment distributed in the cytoplasm responsible for most red, violate, orange and yellow colours found in flowers, fruit, some leaves and underground part of beet root’.

Betalain colourants have been used in a wide variety of food products such as beverages, jams, jellies, ice cream, yoghurt, gelatin desserts, canned fruits, toppings, confections etc. It is a natural food colourant and relatively safe. It has various health benefits. Betalain has no impact on environment. It gives consumers an appeal of fresh foods. The betalain can be used as colourant in organic foods, a developing concept in recent years. Since very low level of colour is used in food product it imparts very less technical defects to product.

Nature identical synthetic colours: These are synthesized in the laboratories and a very limited range is available.

Artificial colours: These are two types FD and C dyes and FD and C lakes. Dyes are water-soluble compounds that produce colour in solution. Lakes are made by combining dyes with alumina to form insoluble colourants. Coal tar is available in wide range of colours. Indigocarmine is an example of synthetic colour.

Inorganic colours: PFA prohibits use of inorganic colour except titanium dioxide, which is permitted in chewing gum (Max limit 1.0 %).

FOOD COLOURS PERMITED BY FSSA

Natural colouring matter which may be used – Except as otherwise provided in the rules the following natural colouring principles whether isolated from natural colours or produced synthetically may be used in or upon any article of food.

Carotenoids
Chlorophyll;

riboflavin(L

actoflavin ;

Caramel;

Annatto;

h) Saffron;

Curcumin or turmeric

Addition of inorganic matter and pigments prohibited- Inorganic colouring matters and pigments shall not be added to any article of food; Provided that chewing gum may contain Titanium dioxide – (food grade) up to a maximum limit of 1 per cent.

Synthetic food colours which may be used- No synthetic food colours or a mixture thereof except the following shall be used in food:

S. No.	Colour	Common Name (1956)	Colour index	Chemical Glass
(1)	(2)	(3)	(4)	(5)
1.	Red	Ponceu 4R	16255	Azo
		Carmoisine	14720	Azo
		Erythrosine	45430	Xanthene
2.	Yellow	Tartrazine	19140	Pyrazolone
		Sunset yellow FCF	15985	Azo

3.	Blue	Indigo Carmine Brilliant Blue FCF	73015 42090	Indigoid Triarylmethane
4.	Green	Fast green FCF	42053	Triarylmethane

Use of Lake colours as colourant in foods—Aluminium Lake of Sunset yellow FCF may be used in powdered dry beverages mix (powdered softdrink concentrate) upto a maximum limit of 0.04 percent weigh by weight. The maximum limit of colour content in final beverage for consumption shall not exceed 8.3 ppm and that of aluminium content shall not exceed 4.4ppm of the final beverage for consumption. Provided that the powdered dry beverages mix (powdered softdrink concentrate) label shall give clear instruction for reconstitution of product for making final beverage.

Toxic trace elements, radionuclides

21.1. INTRODUCTION

The unintentional incorporation of chemicals into food is as widespread as intentional addition and may pose health hazards. The sources of contamination are radioactive fall-out, chemicals used in agricultural production, animal food additives and accidental contaminants during food processing.

21.2. ANTINUTRITIONAL FACTORS

Many foods, particularly those of plant origin, contain a wide range of anti-nutritional factors which interfere with the assimilation of nutrients contained in them. The important anti-nutritional factors are trypsin inhibitors, phytates, oxalates, tannins, lectins and goitrogens. They interfere with the utilization of other nutrients like proteins, minerals like iron, zinc, calcium and iodine.

21.2.1. Trypsin inhibitors

Trypsin inhibitors are proteins distributed widely in plant foods like legumes (soyabean, lima and kidney bean) and certain animal foods like white of egg. They generally inhibit the activity of trypsin in the gut and interfere with digestibility of dietary proteins and reduce their utilization. They are heat labile; the extent and ease of heat inactivation varies from one trypsin inhbitor to another. However, autoclaving at 120^{o}C for 15-30 min inactivates almost all trypsin inhibitors. The heat treatment inactivates the trypsin inhibitors and improve considerably the utilization of protein present in these foods.

21.2.2. Phytate

Phytate is widely distributed in seeds. Unrefined cereals and millets are richest sources of phytates. Phytate is hexa phosphate of inositol. It acts as a source of bound phosphorus for the seeds during germination. These phytates bind iron, zinc, calcium and magnesium. In presence of calcium and magnesium, it forms insoluble complexes with iron and thus makes iron unavailable. Phytates present in cereals contribute significantly to poor absorption of iron from cereal based diets. On germination of the grains, the phytate content reduces due to enzymatic breakdown of phytate. Improved iron availability in germinated grains can be partly attributed to a reduction in phytate content.

21.2.3. Tannins

Tannins are condensed polyphenolic compounds which are widely distributed in plant kingdom. They are present in high amount in seed coat of most legumes, spices, tamarind, turmeric, in certain vegetables and fruits. Millets like bajra, ragi, sorghum also contain a fair amount of tannin. Tannins bind with iron irreversibly and interfere with iron absorption. Tannins are also known to bind proteins and reduce their availability.

21.2.4. Oxalates

Oxalic acids or its salts (oxalates) are widely distributed in plant foods. These oxalates are mostly calcium salts. Rich source of oxalates are green leafy vegetables and green vegetables and some legumes. Oxalates are known to interfere with calcium absorption by forming insoluble salts with calcium. Stone patients are advised to avoid high oxalate containing foods.

21.2.5. Goitrogens

Certain substances present in plant foods interfere with iodine uptake by thyroid gland and may contribute to development of iodine deficiency disorders when iodine intakes are marginal. Such compounds are termed as ‘goitrogens’. Thiocyanate, isothiocyanates and their derivatives etc. These compounds occur in leaves and vegetables like cabbage, cauliflower, rape leaves, radish, rapeseed, mustard, etc. soyabean, peanut, lentils also contain goitrogens.