FOOD CHEMISTRY

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Lesson-1 Structure of 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°.
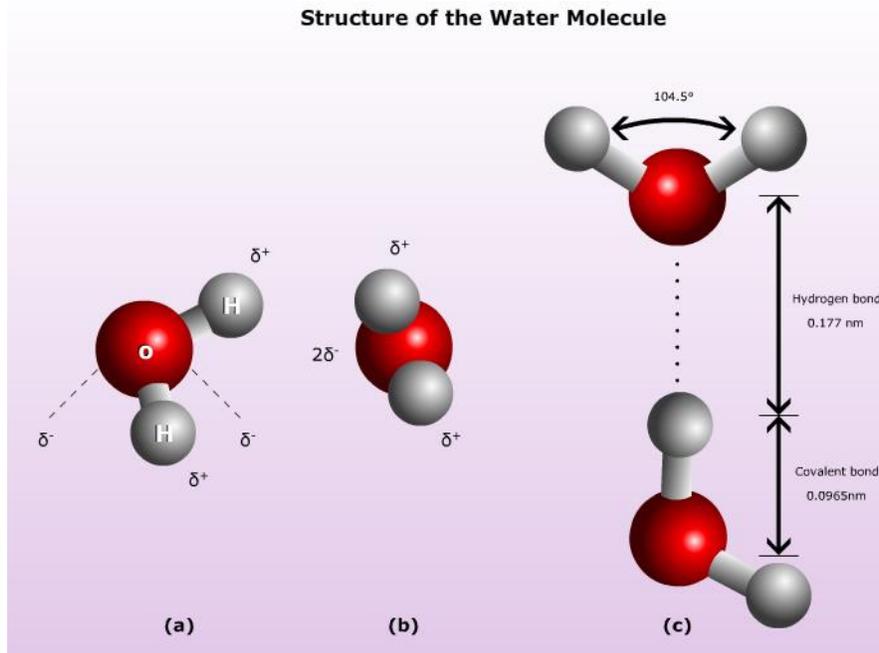
Figure 1.1 Structure of the water molecule
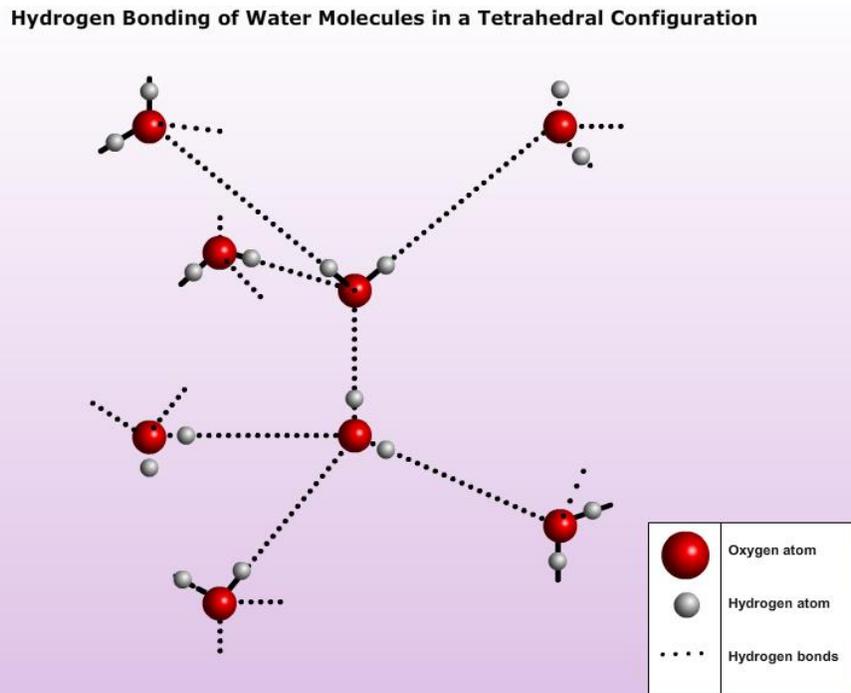
Figure 1.2 Hydrogen bonding of water molecules in a tetrahederal configuration

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.

Structure and Hydrogen-Bond Possiblities for a Hydronium ion
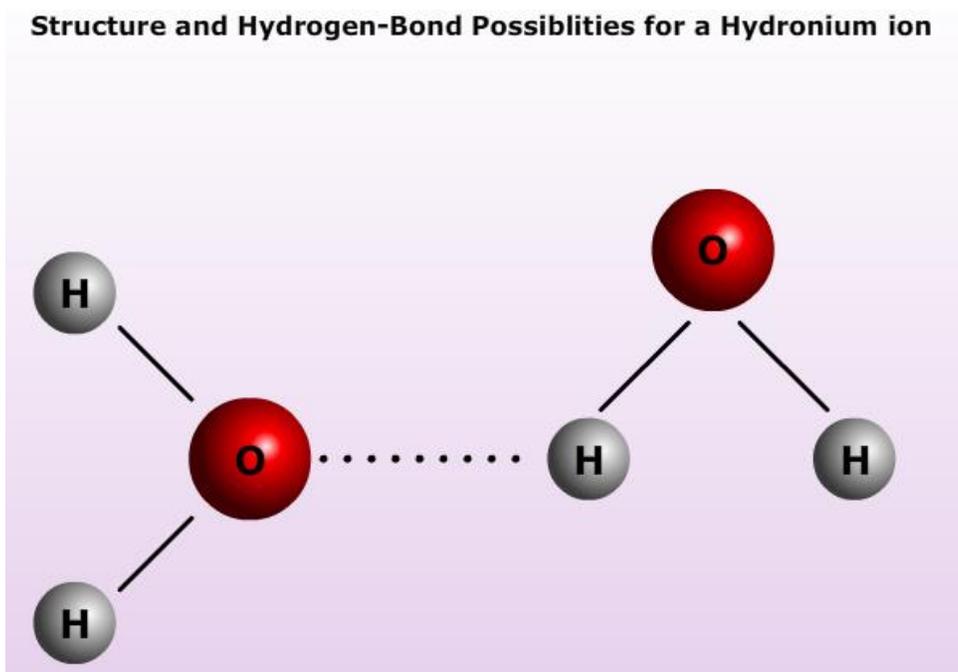
Figure 1.3 Hydronium ion

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.

Hydrogen bonding (dotted lines) of water to two kinds of functional groups occurring in proteins
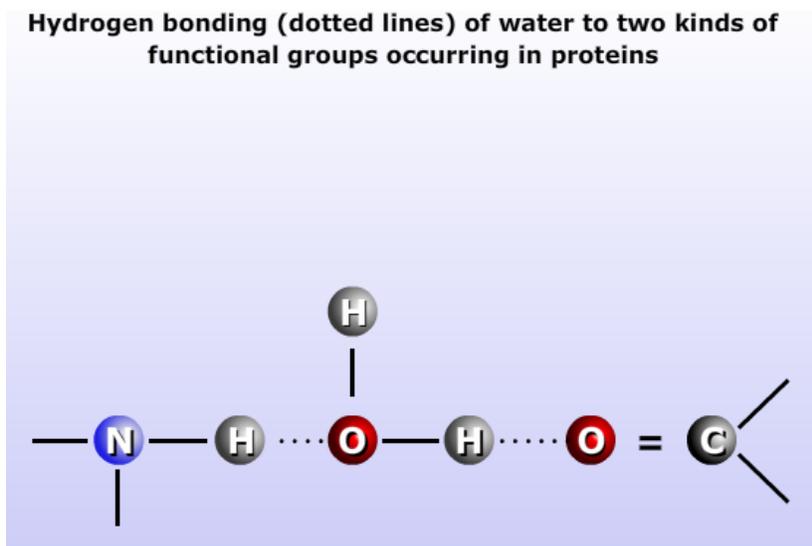
Figure 1.4 Hydrogen bonding of water to two kinds of functional groups occurring in proteins

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.
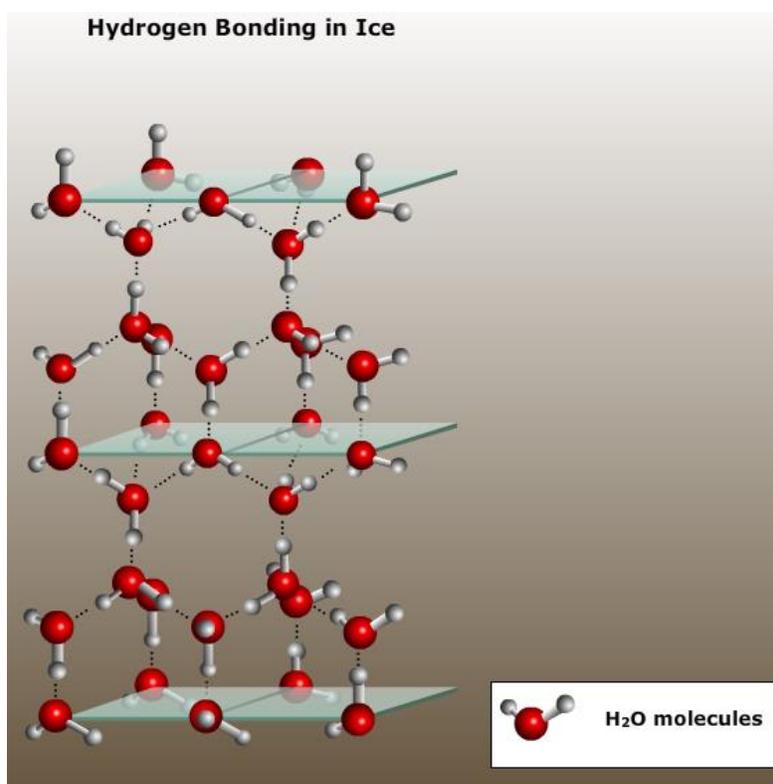
Figure 1.5 Hydrogen bonding in ice

Unit cell of ordinary ice at 0⁰C, circles represent oxygen atoms of water molecules
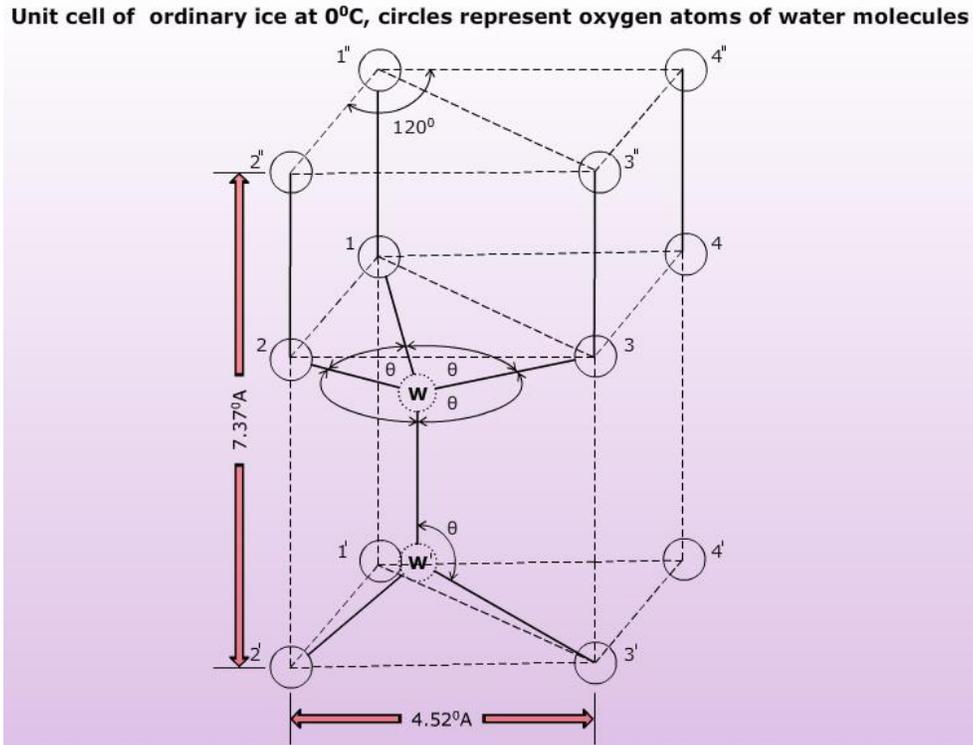
Figure 1.6 Unit cell of ordinary ice

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.

$$*****\ddot\smile*****$$

Module 1 Water

Lesson-2 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.
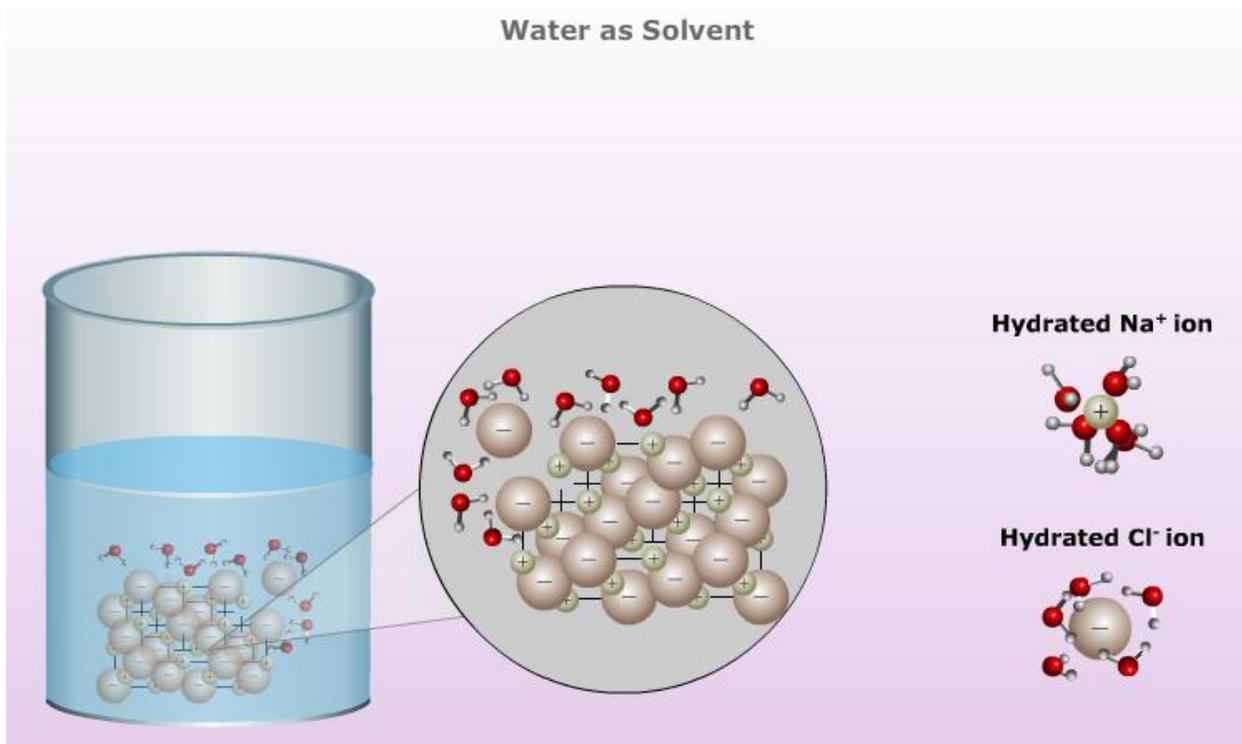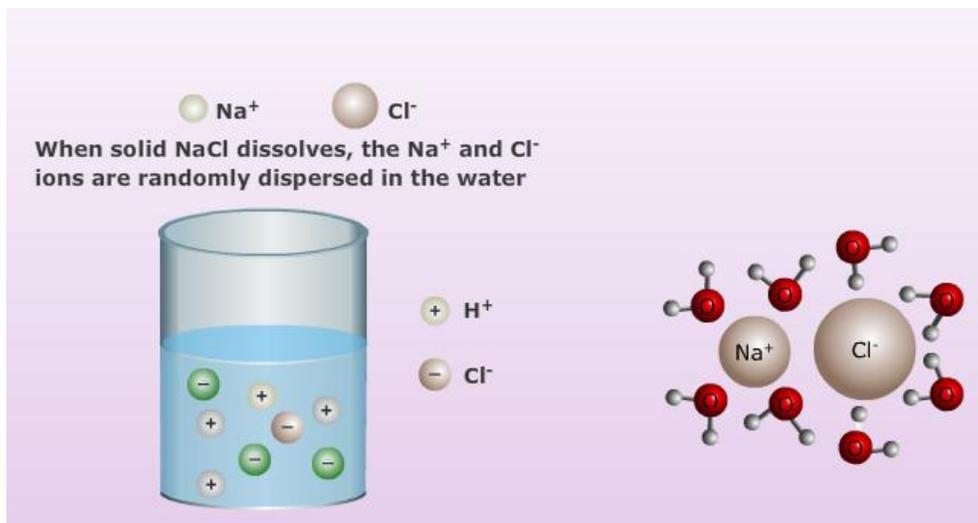
Figure 2.1 Water as solvent

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 non-aqueous 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 water-apolar interfacial area. This process is termed as "hydrophobic interaction".
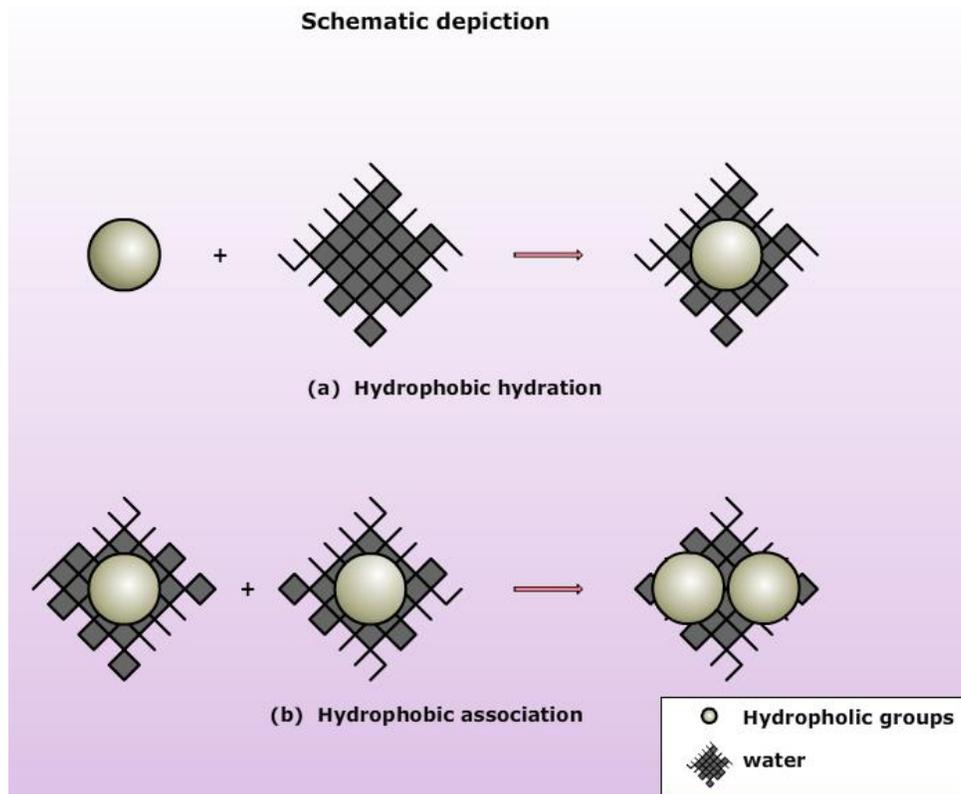
(Figure 2.2 Hydrophobic interaction)
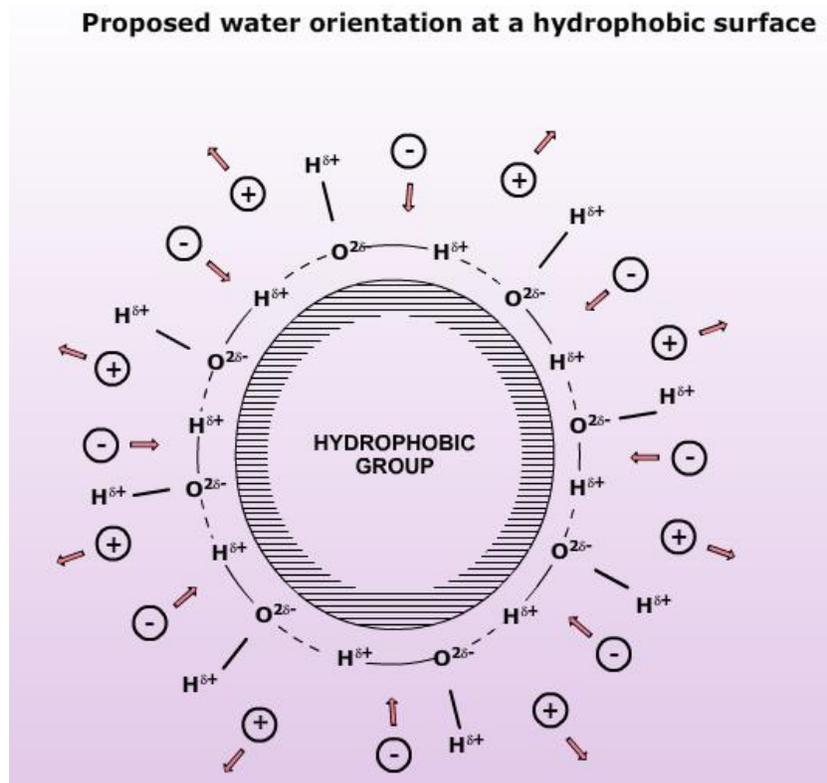
(Figure 2.3 Water orientation at hydrophobic surface)

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.

Schematic depiction of a globular protein undergoing hydrophobic interaction

Hydrophobic groups

Water molecules associated with polar groups
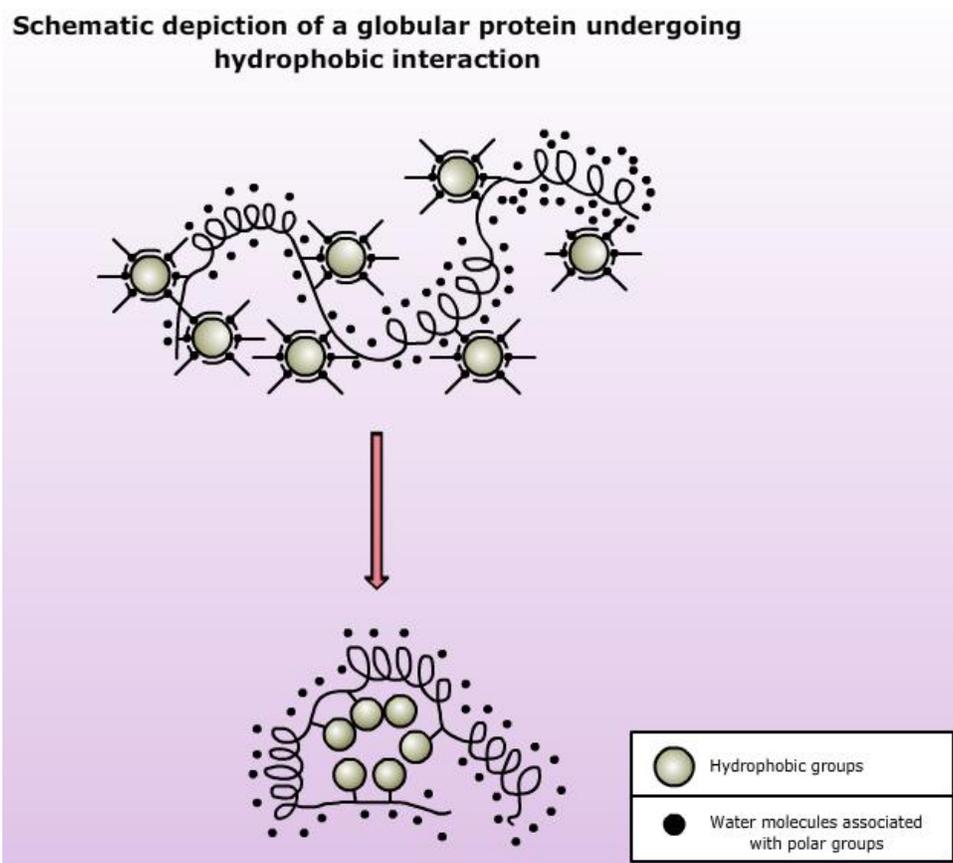
(Figure 2.4 Globular protein undergoing hydrophobic interaction)

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.

2.8. Temperature Dependence

Relative vapor pressure is temperature dependent. The degree of temperature dependence is a function of moisture content. This behavior can be important for a packaged food because it will undergo a change in relative vapour with a change in temperature, causing the temperature dependence of its stability to be greater than that of the same product unpackaged.

2.9. Moisture sorption isotherms

A plot of water content of a food (g water/g dry material) versus a_w at constant temperature is known as a moisture sorption isotherm (MSI).

Information derived from MSIs are useful for concentration and dehydration processes, formulation of food mixtures so as to avoid moisture transfer among the ingredients, determination of moisture barrier properties

needed in a packaging material, determination of what moisture content will curtail growth of microorganisms of interest and prediction of the chemical and physical stability of food as a function of water content.

Resorption (or adsorption) isotherms are prepared by adding water to previously dried samples. Desorption isotherms are isotherms prepared by removing water from samples Isotherms with a sigmoidal shape are characteristic of most foods. Foods such as fruits, confections, and coffee extract that contain large amounts of sugar and other small, soluble molecules and are not rich in polymeric materials exhibit a J-type isotherm.
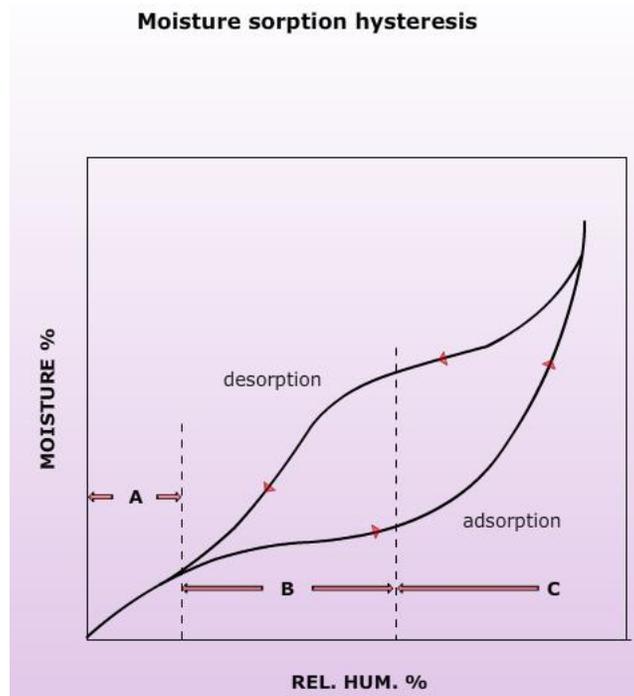
(Figure 2.5 Moisture sorption hysteresis)
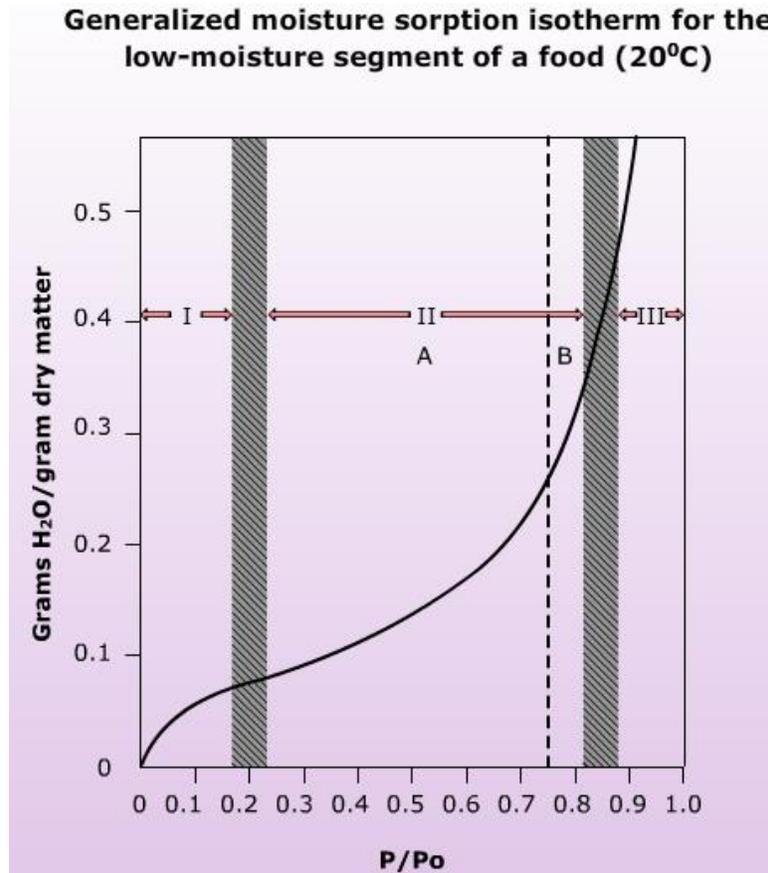
(Figure 2.6 Generalized moisture sorption isotherm)

As water is added (resorption), sample composition moves from Zone I (dry) to Zone III (high moisture). Properties of water associated with each zone differ significantly.

2.9.1. Water in Zone I of the isotherm

The water in Zone I of the isotherm is most strongly sorbed and least mobile, associated with accessible polar sites by water-ion or water-dipole interactions, unfreezable at -40°C, not abile to dissolve solutes, not present in sufficient amount to have a plasticizing effect on the solid, behaving simply as part of the solid and constituting a tiny fraction of the total water in a high-moisture food material.

2.9.2. Water in Zone II of the isotherm

Water in Zone II of the isotherm occupies first-layer sites that are still available, associates with neighboring water molecules and solute molecules primarily by hydrogen bonding, slightly less mobile than bulk water, most of it is unfreezable at - 40°C, exerts a significant plasticizing action on solutes, lowers their glass transition temperatures and causes swelling of the solid matrix. This action, coupled with the beginning of solution

processes, leads to acceleration in the rate of most reactions. Water in Zones I and Zone II usually constitutes less than 5% of the water in a high moisture food material.

2.9.3. Water in Zone III of the isotherm

Water in Zone III of the isotherm causes glass-rubber transition in samples containing glassy regions, very large decrease in viscosity, very large increase in molecular mobility and commensurate increases in the rates of many reactions. This water is referred to as bulk-phase water, having properties of bulk-phase water and will not alter properties of existing solutes, freezable, available as a solvent, readily supports the growth of microorganism and constituting more than 95% of the total water in a high-moisture food. It is the most mobile fraction of water existing in any food sample governs stability.

2.9.4. Hysteresis

An additional complication is that an MSI prepared by addition of water (resorption) to a dry sample will not necessarily be superimposable on an isotherm prepared by desorption. This lack of superimposability is referred to as "hysteresis". The magnitude of hysteresis, the shape of the curves, and the inception and termination points of the hysteresis loop can vary considerably depending on factors such as nature of the food, physical changes it undergoes when water is removed or added temperature, rate of desorption and degree of water removal during desorption.

2.10 Relation of food stability with its water activity

Food stability and its a_w are closely related in many situations. The rates of many reactions are influenced by the extent of water binding in food in which water content is less than TS (<50%). The effect of water activity on processes that influence quality of food is depicted in figure. It is clear that water activity has profound influence on the rate of many chemical reactions in food as well as on the rate of microbial growth.

Relationship Between Water Activity and a Number of Reaction Rates
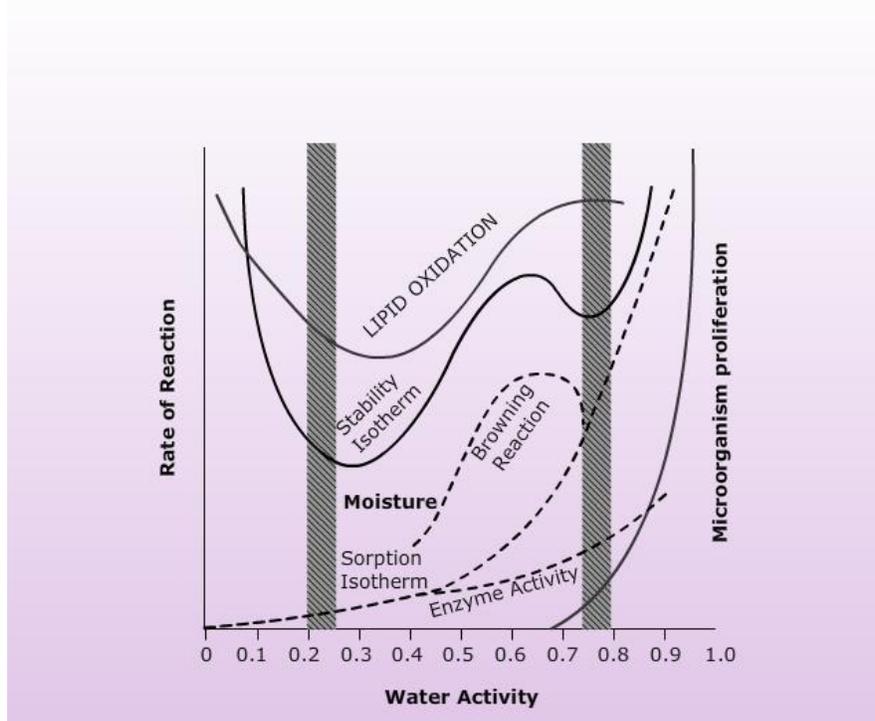
(Figure 2.7 Water activity and a number of reaction rates)

Relationship Between Water Activity and a Number of Reaction Rates
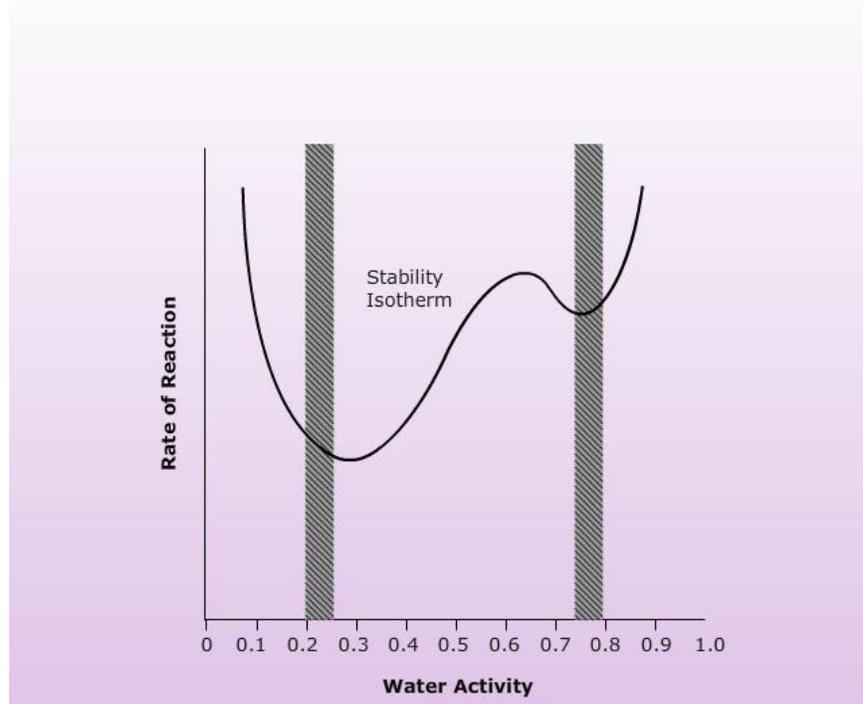
(Figure 2.8 Water activity and a number of reaction rates-stability isotherm)
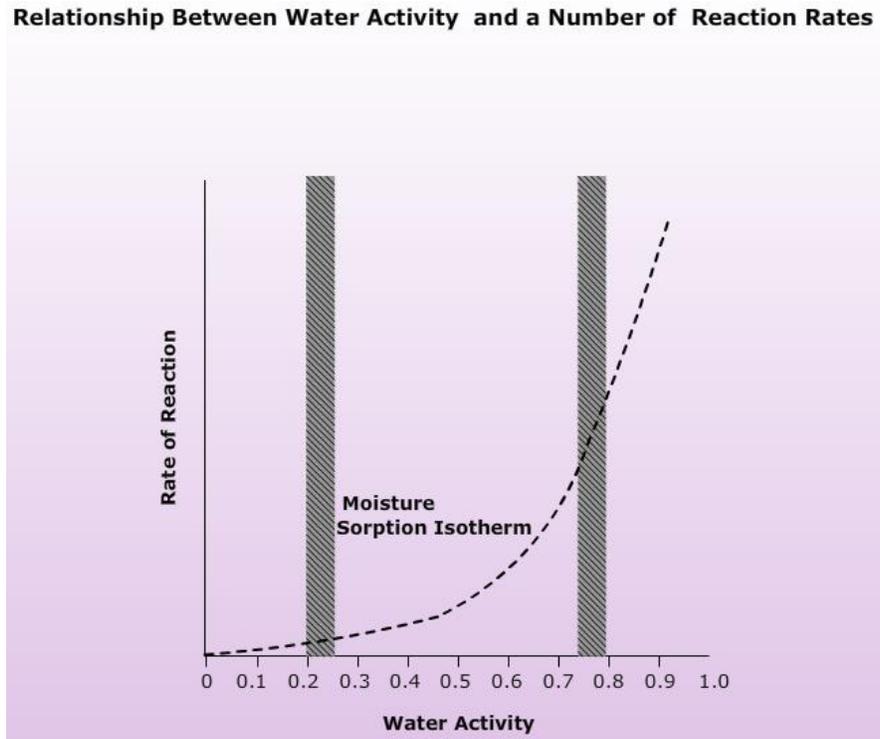
(Figure 2.9 Water activity and a number of reaction rates-moisture sorption isotherm)

Decreased water activity retards growth of microorganisms slows enzyme catalyzed reactions and also retards non-enzymatic browning. Enzyme activity is virtually non-existent in monolayer water (a_w<0.25). Therefore growth of microorganisms at this level of activity is also zero. Mold and yeast start to grow when water activity reaches between 0.7-0.8, which is the upper limit of capillary water. Bacterial growth takes place when water activity reaches 0.8, which is the limit of loosely bound water. However yeast and mold are usually inhibited between 0.8-0.88. Enzyme activity increases gradually between water activity of 0.3-0.6 and than rapidly increases in the loosely bound water range i.e. water activity of about 0.8.
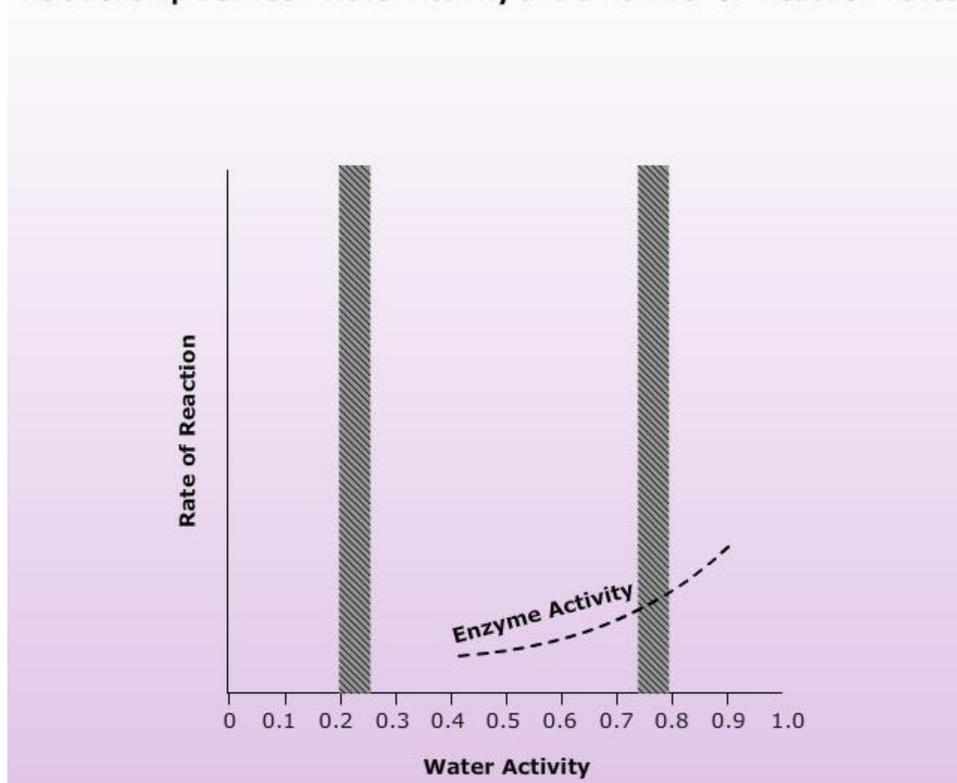
(Figure 2.10 Water activity and a number of reaction rates-enzyme activity)
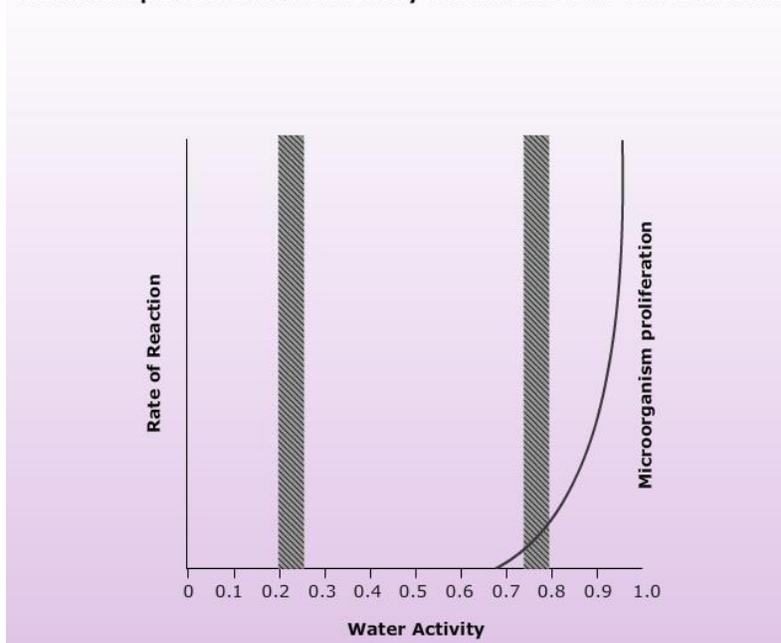
(Figure 2.11 Water activity and a number of reaction rates-microorganism proliferation)

Maillard reaction strongly depends on water activity and reaches a maximum rate at a value of 0.6 to 0.7. Beyond this range the rate of reaction decreases. The explanation for such behaviour is that in intermediate water activity range, the reactants are all dissolved and further increase in a_w leads to dilution of reactants, which adversely affects the reaction rate.

Relationship Between Water Activity and a Number of Reaction Rates

Browning Reaction

Rate of Reaction

0   0.1   0.2   0.3   0.4   0.5   0.6   0.7   0.8   0.9   1.0
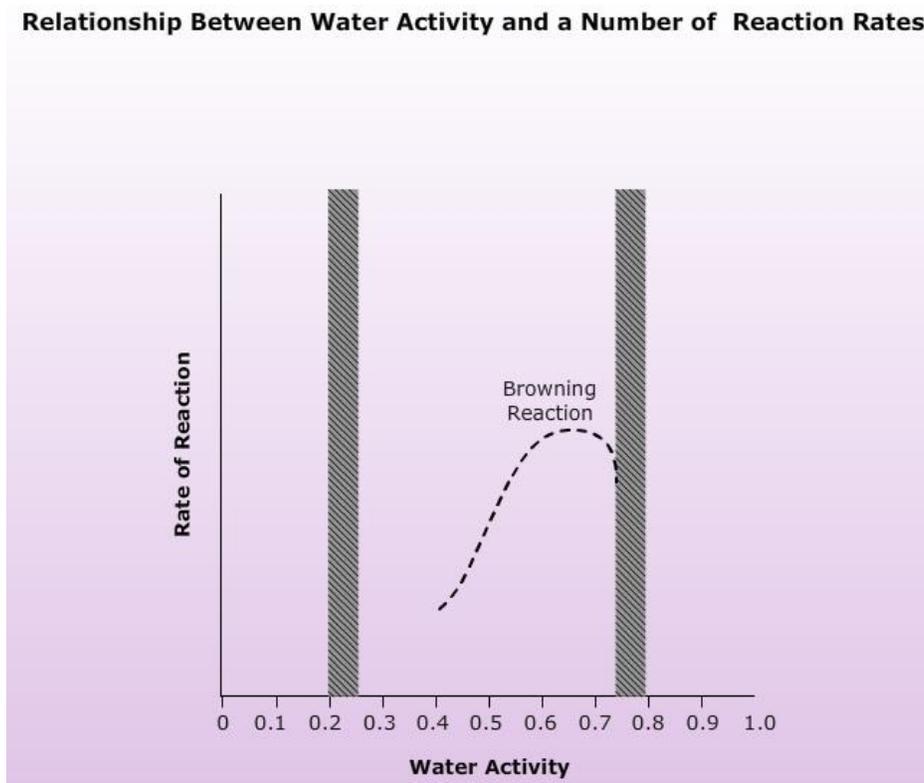
Water Activity

(Figure 2.12 Water activity and a number of reaction rates-browning reaction)

The effect of water activity on oxidation of lipids is complex. Lipid oxidation rates are at a high in the monolayer water range of water activity, reach a minimum at water activity of 0.3-0.4 and then increases to a maximum at 0.8. If we start at very low water activity value, it is apparent that rate of oxidation decreases as water is added. Further addition results in increased rate of reaction followed by another reduction. The interpretation for such a behaviour is that first addition interferes with oxidation probably by—

1. Binding hydroperoxides and thereby interfering with their decomposition which hinders the progress of oxidation.
2. Hydration of metallic ions thereby reducing their effectiveness as catalyst of oxidation
3. Quenching free radicals and by preventing access of O_2 to the lipid which further provides protection against oxidation.

The increases observed by further addition of H_2O maybe due to--

1. Increased solubility of O_2, thereby increasing the mobilization of O_2 as well as catalysts
2. The swelling of macromolecules which exposes more catalytic sites

The second decrease observed at a_w 0.8 may probably be due to dilution of the catalysts that decreases their effectiveness.

Relationship Between Water Activity and a Number of Reaction Rates

Rate of Reaction

LIPID OXIDATION

0   0.1   0.2   0.3   0.4   0.5   0.6   0.7   0.8   0.9   1.0
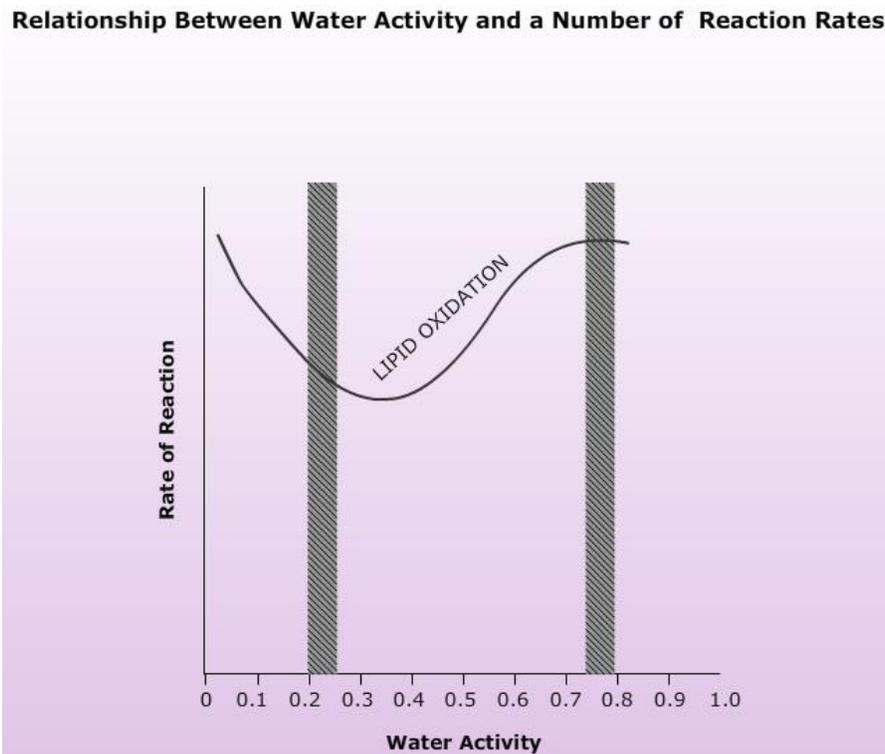
Water Activity

(Figure 2.13 Water activity and a number of reaction rates-lipid oxidation)

Therefore, the storage stability of foods is highest when the a_w lies between 0.2-0.4. Food must be prevented against microbial spoilage when a_w is between 0.6-0.8.

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Lesson -3. Determination of moisture in food

3.1. Introduction

Moisture determination is one of the most important and most widely used measurements in the processing and testing of foods. Since the amount of dry matter in a food is inversely related to the amount of moisture it contains. Therefore it is of direct economic importance to the processor and the consumer. Stability and quality of foods is also affected by moisture content, hence it also has a role in the safety of foods. Grains that contain too much water is subject to rapid deterioration from mold growth, heating, insect damage and sprouting. The rate of browning of dehydrated vegetables and fruits, or oxygen absorption by egg powders increases with an increase in moisture content. High moisture content of ghee leads to hydrolytic rancidity.

Moisture determination is important in many industrial problems, i.e. in the evaluation of materials' balance or of processing losses. Moisture content (and sometimes its distribution) must be known for optimum processing of foods. Milling of cereals, mixing of dough to optimum consistency, and for producing bread with the best grain, texture and freshness retention. Moisture content must be known in determining the nutritive value of food, in expressing results of analytical determinations on a uniform basis, and in meeting compositional standards or laws.

3.2. Some basic considerations

Water may occur in foods in at least three forms.

1. A certain amount may be present as free water in the intergranular spaces and within the pores of the material.

2. Such water retains its usual physical properties and serves as dispersing agent for the colloidal substances and as a solvent for the crystalline compounds.

3. Part of the water is absorbed on the surface of the macromolecular colloids (starches, pectins, cellulose and protein). The water is closely associated with the absorbing macromolecules by forces of absorption (vander waals or hydrogen bond).

4. Finally some of the water is in a bound form in combination with various substances, i.e. water of hydration.

The rapid and accurate determination of water in foods possesses many problems. Many workers have stressed the complexity of analytical procedures for the determination of water in foods. In practice, the guiding principle

has been to prefer the method that gives the highest moisture values, with negligible decomposition volatilization of compounds.

3.3. Methods for determination of moisture in foods

Methods for determination of moisture in food can be divided into four different classes.

1. Drying methods

2. Distillation procedure

3. Chemical assays

4. Physical procedures

3.3.1. Drying methods

The procedures for determination of the moisture content specified in food standards generally involve thermal drying methods. The material is heated under carefully specified temperature and the loss of weight is taken as a measure of the moisture content of the sample. The value obtained for moisture depends on type of oven, temperature and length of drying. Therefore, the methods provide same time approximate rather than accurate moisture values. The rate at which moisture can be removed from the surface of a solid phase is a function water vapour pressure and of the drying temperature. Practical consideration dictates, however, selecting temperatures at which the decomposition of organic compounds is minimised, and yet the time required for quantitative drying at the selected temperature not unduly prolonged.

Advantages

Drying methods, however, are simple, relatively rapid, and permit the simultaneous analyses of large number of samples.

Disadvantages

1. Heating of a moist organic substances causes, in addition, volatilization of material and formation of gaseous product by irreversible thermal decomposition of organic component.

2. Further weight changes resulting from oxidation phenomenon (i.e. oxidation of oils) occur.

3. Improperly maintained dessicator and dessicants can cause erratic results from pick up of moisture during cooling.

4. Formation of a crust that is impervious to evaporation of moisture from the centre of a dried sample.

Factors affecting the precision of moisture measurements by drying methods

The accuracy of moisture determinations is affected by

1. Drying temperature

2. Relative humidity of the drying chamber

3. Air movement in the drying chamber

4. Vacuum in the chamber

5. Depth and particle size of the samples

6. Drying oven construction

7. Number and position of samples in oven

8. Diameter and type of container (material of container)

The rate of evaporation is higher in aluminium than in glass or porcelain dishes, high in vacuum than in simple ovens, and high in shallow than in deep dishes. Solid materials must be pulverised under conditions that minimise compositional changes. In drying liquids, it is essential to spread the material over a large surface. The liquid is preferably evaporated first on a water bath, and then drying is completed in an oven.

To reduce crust formation the sample is moistened with water and thoroughly mixed with sand or asbestos. To increase the area of drying semisolid material is spread with the help of glass rod. Sample weight is generally limited to 3 to 5 g. Standard aluminium dishes are recommended for cereals (55 mm diameter and 15 mm height). The drying temperature used in moisture determination ranges – depending on the tested material – from 70 to 155°C. The average time for drying is from below 1 hr to 6 hr or more. Certain sugars (especially fructose) are sensitive to decomposition at elevated temperatures – in honey and fruit syrups. Fructose solutions decompose at temperature above 70°C and glucose is relatively stable at 98°C. The drying of fructose, glucose or sucrose solutions is faster and the tendency to decompose is less if the pH is below 7.

Foods can be dried for moisture determination either for a selected period of time or until two successive weighing show a negligible loss in weight (generally 1 mg for a 5 g sample, at 20 min interval).Drying time is inversely related to drying temperatures. In foods susceptible to decomposition, drying temperatures can be reduced by using vacuum ovens.

3.3.2. Distillation methods

There are two types of distillation procedures.

a) In one type, water is distilled from an immiscible liquid of high boiling point. The sample suspended in a mineral oil having a flash point much above the boiling point of water is heated to a predetermined temperature in a suitable apparatus. The water that distils off is condensed and is collected in a suitable measuring cylinder.

b) In the second type, the mixture of water and an immiscible solvent (i.e. xylene, toluene, tetrachloroethane) distils off and is collected in a suitable measuring apparatus in which water separates and its volume can be measured.

Liquids with a high specific gravity (tetrachloroethylene, carbon tetrachloride) eliminate fire hazard and reduce the danger of overheating or charring, as sample floats on top of the liquid.

Advantages

Distillation methods cause less decomposition in some foods than drying at elevated temperatures. However, chemical reactions produced by heat are reduced but not eliminated.

Disadvantages

Many difficulties may be encountered in the determination of moisture by the distillation method. These include:

1. Relatively low precision of the receiving measuring device.

2. Difficulties in reading the meniscus.

3. Adherence of moisture droplets to the glass.

4. Overboiling (especially with xylol).

5. Solubility of water in the distillation liquid.

6. Incomplete evaporation of water and underestimation of moisture contents.

7. Distillation of water soluble components.

8. Foods in powder form (cereals, flours, starches) tend to bump during the distillation through overheating on the bottom of the flask.

The main objection to distillation procedures is that they are not adaptable to routine testing. Some of the disadvantages can be overcome by the following interventions

1. Adherence of water to the walls of the condenser tubes or sides of the receiving tubes can be generally remedied by using thoroughly cleaned glassware.

2. Use of small amount of wetting agent will also improve meniscus reading.

3. Incomplete recovery of water due to the formation of an emulsion can sometimes be remedied by adding small amounts of amyl alcohol or isobutyl alcohol.

4. To improve the moisture distillation, wide mouthed boiling flasks can be used.

5. Dispersing the tested material on diatomaceous earth or on filter – cel is useful with many viscous foods rich in sugar or protein.

6. Bumping of powder foods can be overcome through the introduction of a small amount of dry short fiber asbestos.

7. Adverse effects of heat can be reduced still further by selecting organic solvents with a boiling point below that of water, such as benzene. Such a choice, however, lengthens the distillation time.

8. For accurate results, standard apparatus and careful attention to specified procedures are essential.

3.3.3. Chemical methods

Karl Fischer Reagent Titration

The waretime need for dehydrated foods stimulated the search for more rapid and accurate methods for determining moisture. The Karl Fischer reagent has proved to be quite adaptable for this purpose.

Advantages

It is a method of choice for determination of water in many low moisture foods such as dried fruits and vegetables, candies, chocolate, roasted coffee, oils and fats.

Superiority of the method was demonstrated in determining moisture in sugar rich foods (honey) or foods rich both in reducing sugars and proteins.

The procedure has been applied also to foods with intermediate moisture foods (bakery doughs, baked products, fat rich cake mixes) and to foods with high levels of volatile oils.

The Karl Fischer reagent has proved to be quite adaptable for moisture determination by chemical method.

The Karl Fischer method for moisture determination is based on the reaction which involves the reduction of iodine by sulphur dioxide in the presence of water.

$$2 H_2O + SO_2 + I_2 ------------- H_2SO_4 + 2 HI$$
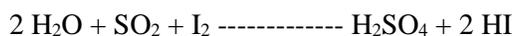
As shown by the above reaction for each mole of water one mole of iodine is required.

Methanol is used to dissolve iodine and pyridine is used to dissolve sulphur dioxide.

Numerous variations have been proposed for the preparation of the Fischer reagent.

The sample in which water is to be determined is dispersed in an appropriate solvent (i.e. methanol, mixture of methanol-sulphur dioxide-pyridine etc.).

The solution is then titrated with a solution of iodine in methanol.

The excess of iodine that cannot react with water is in free form.

Adding to the system a few drops of methylene blue gives a green end point.

Interfering substances are ascorbic acid (oxidation), aldehydes, ketones (release water), mercaptans, diacylperoxide, thioacids and hydrazines – fading end point.

The determination of moisture is carried out in a non aqueous system.

Fluids are delivered but with an automatic pipet or syringe.

Viscous fluids or pastes are generally homogenized with a solvent.

Solids are either homogenized with solvent or titrated as suspensions.

Granular products must be pulverized.

Disadvantages:

The main difficulty in using the Karl Fischer method arises from the lack of complete water extraction.

Formaldehyde is found to be a more rapid and versatile extractant of water from foods than methanol.

Modification of the extraction procedure is exemplified by a method for the water determination in dairy products, where in xylene or carbon tetrachloride is employed in mixed solvent systems with alcohol.

3.3.4. Physical Methods

1) Infrared determination – based on measuring the absorption at wavelengths

 characteristic of the molecular vibration in water. The most useful wavelengths are 3.0

 & 6.1 μm.

2) Gas chromatographic method: based on extracting the moisture with an organic

 solvent and determining water in the extract by gas chromatography.

3) Nuclear magnetic resonance

4) Electric method

5) Densitometric method

6) Refractometric method

7) Polarimetric method

$$*****\bigodot*****$$

Lesson-4 **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 D-line of sodium are always negative and for globular proteins the values of $[\alpha]^{D}_{20}$ are usually within the range of -30° to -60°. 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.

$$\ast\ast\ast\ast\ast \odot \ast\ast\ast\ast\ast$$

Lesson-5   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 (60°-90°C/1 h or less).

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

The predenatured transition state involves minor conformational changes that occur prior to denaturation. As the reaction proceeds, changes due to denaturation occur. Following these changes, the protein may react

either with themselves and/or with other food constituents resulting in the formation of higher molecular weight aggregates. These post-denaturation reactions are virtually irreversible.

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

Digestibility is often improved. In general denatured proteins are more readily attacked by proteolytic enzymes.

Several enzymes like proteases, lipoxygenases, polyphenol oxidases, etc. are inactivated. This limits the undesirables changes like development of off-flavours, acidity, textural changes and discoloration of foods during storage.

Proteinaceous anti-nutritional factors present in seeds and legumes are denatured and inactivated by mild heat treatments. These inhibitors impair efficient digestion of proteins and thus reduce their bioavailability.

Certain proteinaceous toxins, e.g. botulism toxin and enterotoxins are inactivated.

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

5.2.2. Desulfuration: Thermal treatments of proteins or proteinaceous foods at high temperature and in the absence of any added substances can lead to several chemical changes. Most of these chemical changes are irreversible and some of these reactions result in the formation of amino acid types that are potentially toxic. One of the first noticeable changes in proteins on heating at around 100ºC 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 115ºC 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 100ºC. 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 200ºC 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 200ºC.

Since D-amino acids have no nutritional value, racemization of an essential amino acid reduces its nutritional value by 50%. Racemization of amino acid residues causes a reduction in digestibility because peptide bonds involving D-amino acid residues are less efficiently hydrolyzed by gastric and pancreatic proteases. This leads to loss of essential amino acids that have racemized and impairs the nutritional value of the protein. D-amino acids are also less efficiently absorbed through intestinal mucosal cells and even if absorbed they can't be utilized in in-vivo protein synthesis.

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

Formation of Dehydroalanine

$$HOOC-\overset{\overset{\displaystyle NH_2}{|}}{CH}-CH_2-S-S-CH_2-\overset{\overset{\displaystyle NH_2}{|}}{CH}-COOH \qquad \text{Cystine}$$

$\downarrow OH^-$

$$HOOC-\overset{\overset{\displaystyle NH_2}{|}}{CH}-CH_2-S-S-CH_2-\overset{-}{C}-COOH \qquad \text{Carbanion Derivative Of Cystine}$$

$$\downarrow$$

$$CH_2=\overset{\overset{\displaystyle COOH}{|}}{C}-\underset{\underset{\displaystyle NH_2}{|}}{} \quad + \quad HOOC-\overset{\overset{\displaystyle }{|}}{CH}-CH_2-S-S^-$$
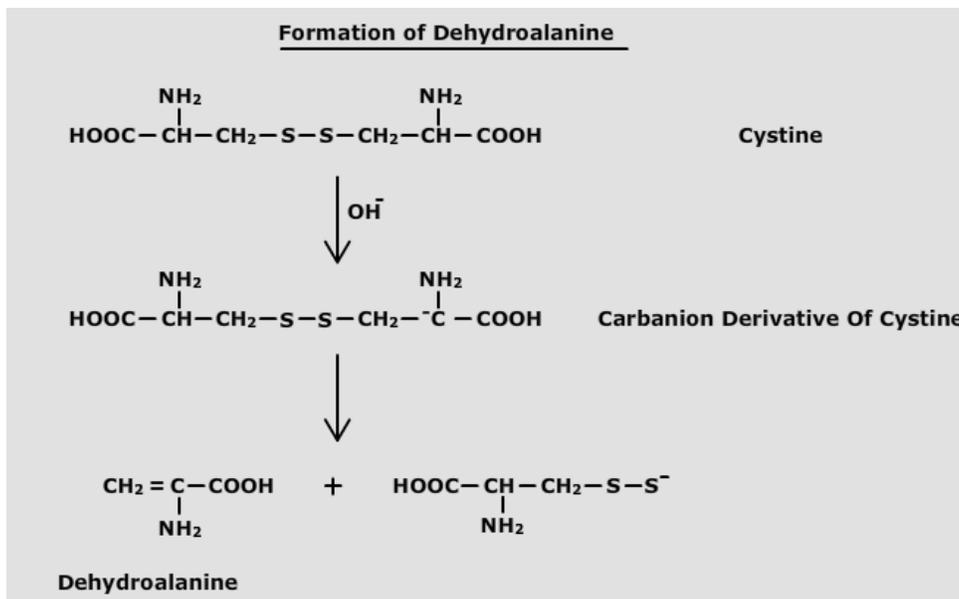
Dehydroalanine

(Figure 5.1 Formation of Dehydroalanine)

Formation Of Lysinoalanine

$$NH_2-\overset{\underset{\displaystyle COOH}{|}}{CH}-(CH_2)_4-NH_2 \; + \; CH_2=\overset{\underset{\displaystyle NH_2}{|}}{C}-COOH \longrightarrow NH_2-\overset{\underset{\displaystyle COOH}{|}}{CH}-(CH_2)_4-NH-CH_2-\overset{\underset{\displaystyle NH_2}{|}}{CH}-COOH$$

Lysine   Dehyroalanine   Lysinoalanine
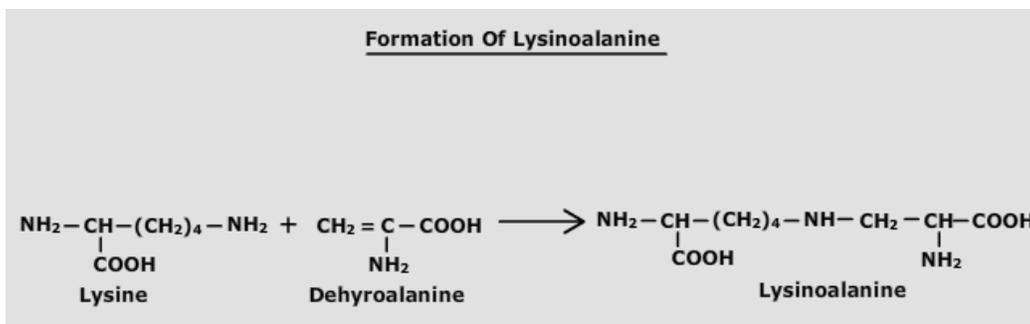
(Figure 5.2 Formation of Lysinoalanine)

Formation Of Ornithoalanine

$$NH_2-\overset{\underset{\displaystyle COOH}{|}}{CH}-(CH_2)_3-NH_2 \; + \; CH_2=\overset{\underset{\displaystyle NH_2}{|}}{C}-COOH \longrightarrow NH_2-\overset{\underset{\displaystyle COOH}{|}}{CH}-(CH_2)_3-NH-CH_2-\overset{\underset{\displaystyle NH_2}{|}}{CH}-COOH$$

Ornithine   Dehydroalanine   Ornithoalanine
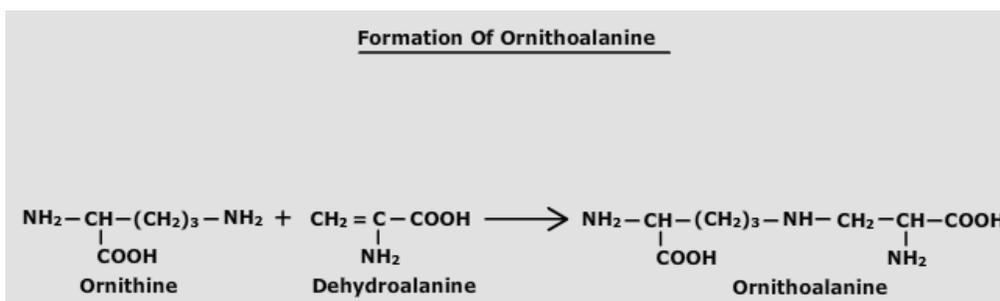
(Figure 5.3 Formation of Ornithoalanine)

Formation Of Lanthionine

$$NH_2-CH-CH_2-SH \ + \ CH_2=C-COOH \longrightarrow NH_2-CH-CH_2-S-CH_2-CH-COOH$$

Cysteine   Dehydroalanine   Lanthionine

(with COOH below the first CH, NH₂ below C; and COOH, NH₂ below the product chain)
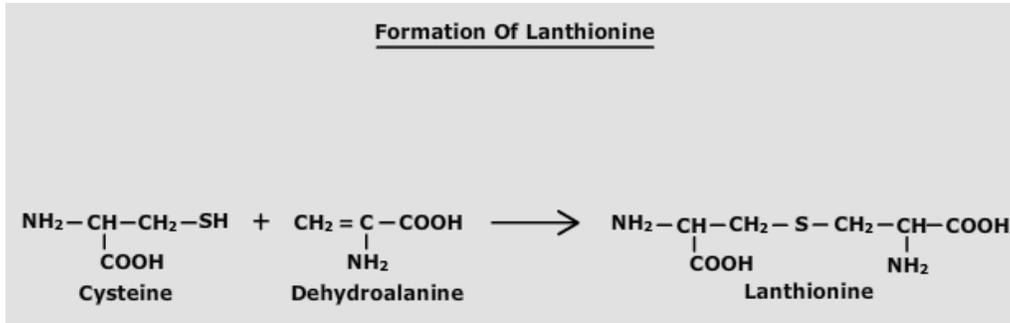
(Figure 5.4 Formation of Lanthionine)

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.

Sugar Amine Condensation

D - Glucose   Schiff's Base   N - Substituted Glycosylamine
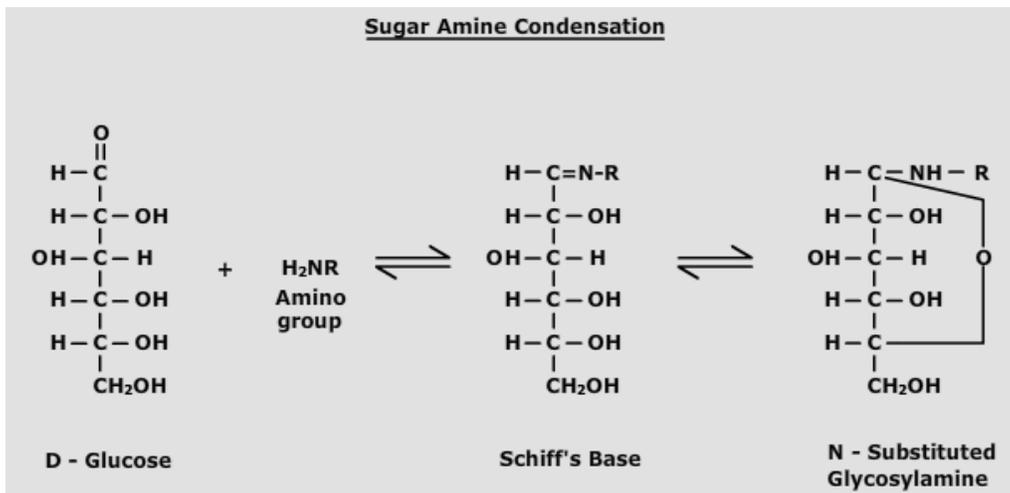
(Figure 5.5 Sugar Amine Condensation)

(Figure 5.6 Amadori Rearrangement)

(Figure 5.7 Degradation of Amadori Compounds)
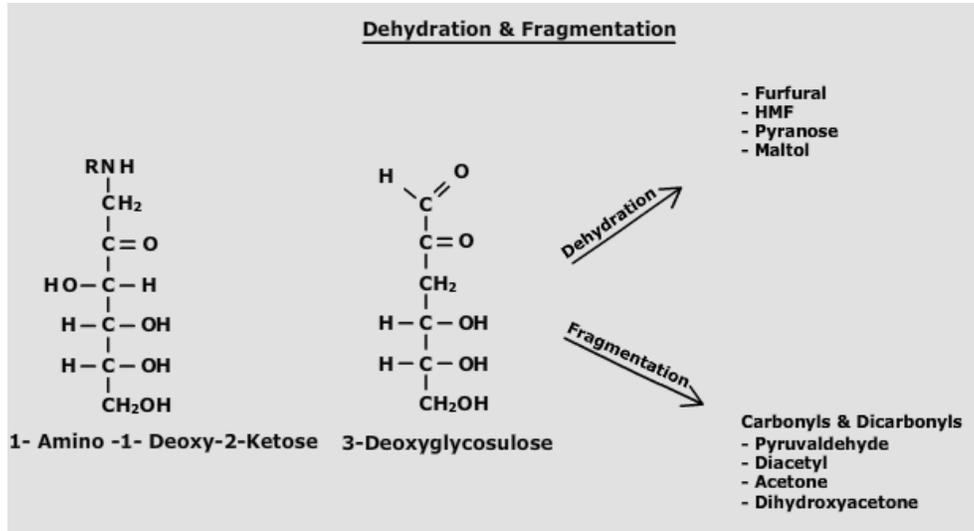
(Figure 5.8 Dehydration and Fragmentation)
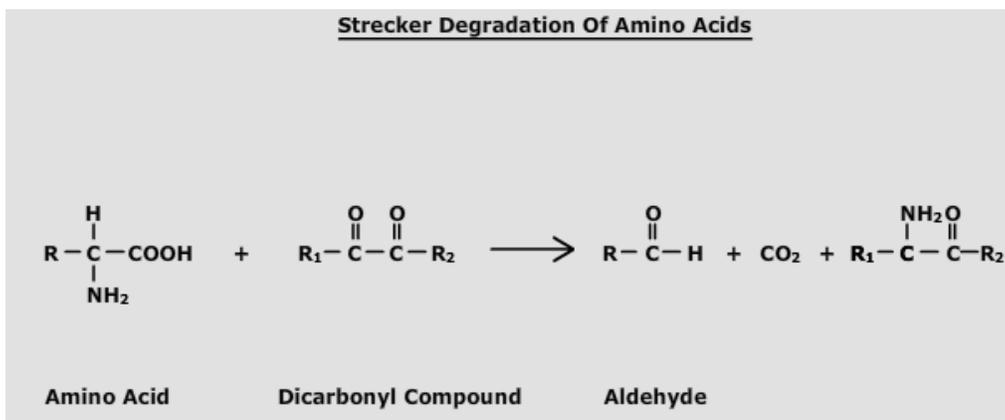
(Figure 5.9 Strecker Degradation of Amino Acids)

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.

Lesson-6 Enzyme catalyzed reactions involving hydrolysis and proteolysis

6.1. INTRODUCTION

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

1. **Peptidases (exopeptidases) -** These enzymes cleave amino acids or dipeptide in a 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-κ-casein 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

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Lesson-7   Theories of formation of texturized proteins

7.1. Introduction

Texturization (when applied to plant proteins) is the development of a physical structure which will provide, when eaten, a sensation of eating meat. Meat "texture" is a complex concept comprising visual aspect (visible fibres), chewiness, elasticity, tenderness and juiciness. The principal physical elements of meat which create the texture complex are: the muscle fibres and the connective tissue. Many plant proteins have globular structure. Texturization confers a fiber-like structure to globular proteins. Suitable processes give the protein chewiness and good water holding property, cooking strength and a meat-like structure. These products have an ability to retain these properties during subsequent hydration and heat treatment. These texturized proteins are often used as meat substitutes, extenders and meat analogues. Commercial textured vegetable protein products are manufactured almost exclusively from soy protein.

7.2. Process of Texturization

The more successful approaches to plant protein (soy protein) texturization can be classified in two categories. The first approach tries to assemble a heterogeneous structure comprising a certain amount of protein fibres within a matrix of binding material. The fibres are produced by a "spinning" process, similar to that used for the production of synthetic fibres for the textile industry. The second approach converts the soy material into a hydratable, laminar, chewy mass without true fibres. Two different processes can be used to produce such a mass: thermoplastic extrusion and steam texturization**.** During texturization, globular proteins are unfolded by the breaking of intra-molecular binding forces. The resultant extended protein chains are stabilized through interactions within the neighbouring chains.

7.2.1. Spin Process/Fiber Spinning

In this method the starting material should contain 90 % or more protein (protein isolate). The molecular weight of proteins should be in the range of 10-50 kdal. Proteins of less than 10 kdal are weak fiber builders, while those higher than 50 kdal are disadvantageous due to their viscosity and tendency to gel in the alkaline pH range. The major steps involved are

A solution of high protein concentration (10-40%) is prepared. The viscous concentrated protein solution is technically known as dope. The protein is then solublized by addition of alkali by raising pH of the dope to about 10. The dope is aged at this high pH with continuous stirring. At such a high pH, the electrostatic

repulsions promote complete dissociation of the proteins into sub units and also causes extensive unfolding of the individual polypeptide chain. All this results in high viscosity. However, prolonged exposure to the high pH should be avoided to minimize the loss of sulfur containing amino acids and to avoid formation of potentially toxic degradation products.

The dope is then pressed through a die-plate containing a thousand or more holes each with a diameter of 50-150 μm. As the dope flows through these holes, streaming orientation of the unfolded protein molecules takes place. Thus the molecules tend to extend and align themselves in a parallel manner.

The liquid filaments coming out of the die enter a coagulation bath at pH 2-3. This bath contains an acid (acetic, citric, phosphoric, lactic, or hydrochloric) and usually 10 % NaCl. Here the proteins are coagulated by the iso-electric pH and by salting-out effect. Because of their elongated, parallel orientation the protein molecules of each filament interacts strongly with each other through hydrogen, ionic and disulfide bonds, to form a hydrated protein fiber.

The coagulated protein fibers are removed from the bath on rollers, in a winding-up state. The speed of the roller is such that the fibers on the rollers get stretched and as a result the individual polypeptide chains achieve still better alignment, associate more closely and form more intermolecular bonds. This increases the mechanical strength and chewiness of the fiber, but may decrease their water holding capacity.

The fibers are then compressed with /without heating between rollers to remove some water, promote adhesion and increase toughness. The bundles are then placed in a neutralizing bath (NaHCO$_3$ and NaCl) at pH 5.5 to 6.0. Sometimes it may also be placed in a hardening bath of NaCl.

Additional treatments involve passage of the fibers through a bath containing a binder and other additives such as aroma compounds and lipids. This improves the thermal stability and aroma.

Finally, the soaked fiber bundles are heated, cut, assembled and compressed. These fibers are similar to those found in meat and the texture of products containing spun fibers resembles meat.

7.2.2. Extrusion Method/Thermoplastic Extrusion

This is the major technique used at present for texturization of vegetable proteins and is also referred to as thermoplastic extrusion. It leads to the formation of dry, fibrous, porous granules or chunks, which possess a chewy texture upon rehydration. The starting material for these processes need not be a protein isolates. Thus, less costly protein concentrates or flours (containing 45-70% protein) can be used. The addition of small amounts of starch or amylose improves the final texture but a lipid content of above 5-10% is detrimental. Up to 3% NaCl, CaCl$_2$ may also be added to improve the texture. The major **steps** involved are-

The moisture content of the starting material is adjusted to 30-40% and the additives are incorporated.

The protein mixture is fed to the extruder where it is exposed to a high pressure(10,000 to 20,000 kPa)

Over a period of 20-150 s, the mixture is elevated to a temperature of 150-200ºC. Under these conditions, the mixture is transformed into a plastic viscous state, in which solids are dispersed. Hydration of the proteins takes place after partial unfolding of the globular proteins followed by stretching and rearrangement of the protein strands along the direction of mass transfer. The thermal coagulation of proteins may also occur.

The mixture is then extruded through a small diameter orifice into normal pressure environment. This results in flash evaporation of the internal water with the formation of expanding steam bubbles leaving behind vacuoles in the protein chunks.

After cooling, the protein polysaccharide matrix possesses a highly expanded dry structure. The porous material is able to absorb 2 to 4 times its weight of water giving a fibrous, spongy structure with chewiness like meat. These products are stable even under sterilization conditions.

$$***** \ddot\smile *****$$

Module 3. Food Lipids

Lesson-8: Edible 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.

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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.
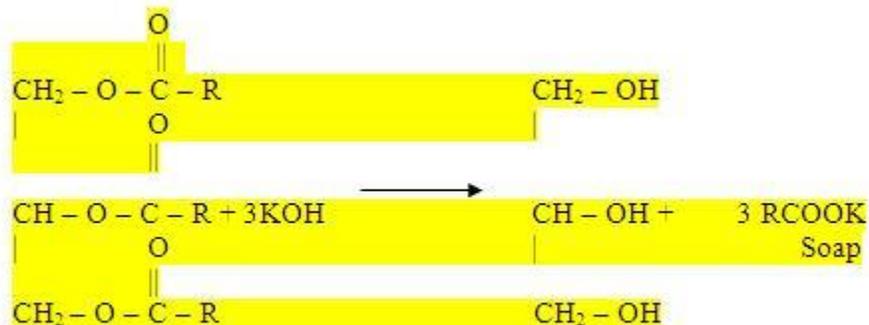
Fig. 8.1: Saponification reaction

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 (1-7g/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 β-sitosterol. 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

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Lesson-9
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%.

Lesson 10
Lipoprotein – definition, classification and involvement in the formation of biological membranes.

10.1. INTRODUCTION

Lipoproteins are aggregates, consisting of proteins, polar lipids and triacylglycerols; which are water soluble and can be separated into protein and lipid moieties by an extraction procedure using suitable solvents. This indicates that only non-covalent types of bonds are involved in the formation of lipoproteins. The aggregates are primarily stabilized by hydrophobic interactions between the apolar side chains of hydrophobic regions of the protein and the acyl residues of the lipid. In addition, there is a contribution to stability by ionic forces between charged amino acid residues and charges carried by the phosphatides. Hydrogen bonds play a small role in binding lipids molecule as there are only few sites available for such linkages. In wheat flour, the lipoprotein complex consists of prolamine and glutelin attached to glycolipids by hydrogen bonds and hydrophobic forces. Thus, lipoproteins are held together only by non-covalent bonds.

10.2. CLASSIFICATION

Lipoproteins exist as globular particles in an aqueous medium. They are solubilized from biological sources by buffers with high ionic strength, by a change of pH or by detergents in the isolating medium. The latter, a more drastic approach, is usually used in the recovery of lipoproteins from membranes. Lipoproteins are characterized by ultracentrifugation. Since lipids have a lower density (0.88–0.9 g/ml) than proteins (1.3–1.35 g/ml), the separation is possible because of differences in the ratios of lipid to protein within a lipoprotein complex.

The lipoproteins of blood plasma have been thoroughly studied. They are separated by a stepwise centrifugation in solutions of NaCl into three fractions with different densities.
A. The "very low density lipoproteins" (VLDL): The density of these types of lipoproteins is <1.006 g/ml): The VLDL fraction can be separated further by electrophoresis into chylomicrons (the lightest lipoprotein, density <1.000 g/ml) and pre-β-lipoprotein.
B. The "low density lipoproteins" (LDL): The density of LDL is 1.063 g/ml. Lipoproteins in the LDL fraction from an electrophoretic run have mobility close to that of blood plasma β-globulin. Therefore, the LDL fraction is denoted as β-lipoprotein.
C. The "high density lipoproteins" (HDL): The density of HDL is 1.21 g/ml. An analogous designation of α-lipoprotein is assigned to the HDL fraction.

Chylomicrons, the diameters of which range from 1000–10,000Å, are small droplets of triacylglycerol stabilized in the aqueous medium by a membrane-like structure composed of protein, phosphatides and cholesterol. The role of chylomicrons in blood is to transport triacylglycerols to various organs, but preferentially from the intestines to adipose tissue and the liver. The milk fat globules have a structure similar to that of chylomicrons. Certain diseases related to fat metabolism (hyperlipidemias) can be clinically diagnosed by the content and composition of the plasma lipoprotein fractions. Electron microscopy studies have revealed that the fat globules in milk have small particles attached to their membranes; these are detached by detergents and have been identified as LDL.

10.3. INVOLVEMENT IN THE FORMATION OF BIOLOGICAL MEMBRANES

Membranes that compartmentalize the cells and many subcellular particles are formed from two main building blocks: proteins and lipids (phospholipids and cholesterol). The glycerophospholipids are the main structural component of biological membranes, such as the cellular plasma membrane and the intracellular membranes of organelles; in animal cells the plasma membrane physically separates the intracellular components from the extracellular environment. The glycerophospholipids are amphipathic molecules (containing both hydrophobic and hydrophilic groups) that contain a glycerol core linked to two fatty acid-derived "tails" by ester linkages and to one "head" group by a phosphate ester linkage. While glycerophospholipids are the major component of biological membranes, other non-glyceride lipid components such as sphingomyelin and sterols (mainly cholesterol in animal cell membranes) are also found in biological membranes. In plants and algae, the galactosyl diacylglycerols, and sulfoquinovosyl diacylglycerols, which lack a phosphate group, are important components of membranes of chloroplasts and related organelles and are the most abundant lipids in photosynthetic tissues, including those of higher plants, algae and certain bacteria. Differences in membrane structure and function are reflected by the compositional differences of membrane proteins and lipids. Studies of membrane structure are difficult since the methods for isolation and purification profoundly change the organization and functionality of the membrane.

Model membranes are readily formed. The major forces in such events are the hydrophobic interactions between the acyl tails of phospholipids, providing a bilayer arrangement. In addition, the amphipathic character of the lipid molecules makes membrane formation a spontaneous process. The acyl residues are sequestered and oriented in the non-polar interior of the bilayer, whereas the polar hydrophilic head groups are oriented toward the outer aqueous phase.

Another arrangement in water that satisfies both the hydrophobic acyl tails and the hydrophilic polar groups is a globular micelle. Here, the hydrocarbon tails are sequestered inside, while the polar groups are on the surface of the sphere. There is no bilayer in this arrangement hydrophobic lipid tails
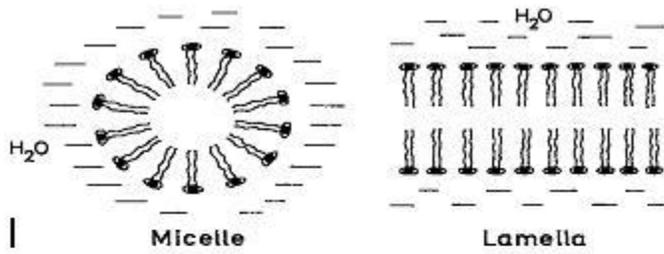
Fig-10.1: Arrangement of polar acyl lipids in aqueous medium.
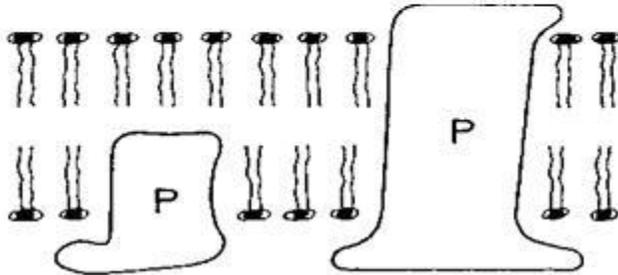
Fig-10.2: Fluid mosaic model of a biological membrane.

The favored structure for most phospho- and glycolipids in water is a bimolecular arrangement, rather than a micelle. Globular proteins, often including enzymes, are found in animal cell membranes and are well embedded or inserted into the bimolecular layer. Some of these so-called integral membrane proteins protrude through both sides of the membrane (fluid mosaic model). Although integral proteins interact extensively with the hydrophobic acyl tails of membrane lipids they are mobile within the lipid membrane.

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Lesson-11

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	$\alpha, 1 \rightarrow 4$	Linear	amylose	Starchy material
	$\alpha, 1 \rightarrow 4$ $\alpha, 1 \rightarrow 6$	Branched	amylopectin	Starchy material
	$\alpha, 1 \rightarrow 4$ $\alpha, 1 \rightarrow 6$	Branched	glycogen	Animal liver
	$\beta, 1 \rightarrow 4$	Linear	Cellulose	Cell walls of all plants
	$\beta, 1 \rightarrow 3$ $\beta, 1 \rightarrow 4$	Linear	β - glucan	Cereal grains (oats, barley)
Fructans	$\beta, 2 \rightarrow 6$ $\beta, 2 \rightarrow 1$	Branched	Fructans	Various plants (wheat endosperm)
	$\beta, 2 \rightarrow 1$	Linear	Inulin	Jerusalem artichokes
Arabinans	$\alpha, 1 \rightarrow 3$ $\alpha, 1 \rightarrow 5$	Branched	Pectic substances	Sugar beet, citrus pectins
Xylans	$\beta, 1 \rightarrow 4$	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.)

$$*****\bigodot*****$$

Lesson-12

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.
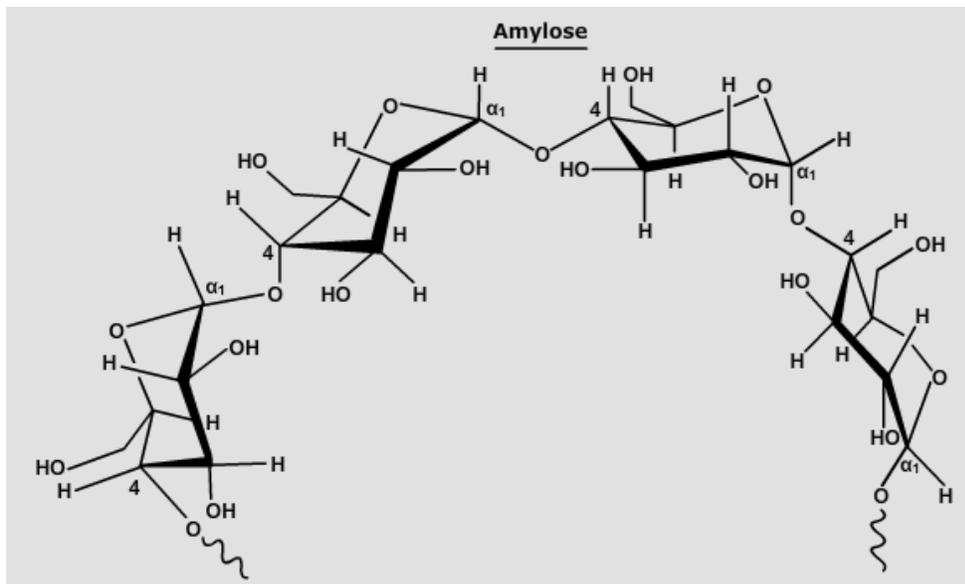
(Figure 12.1 Amylose)

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.
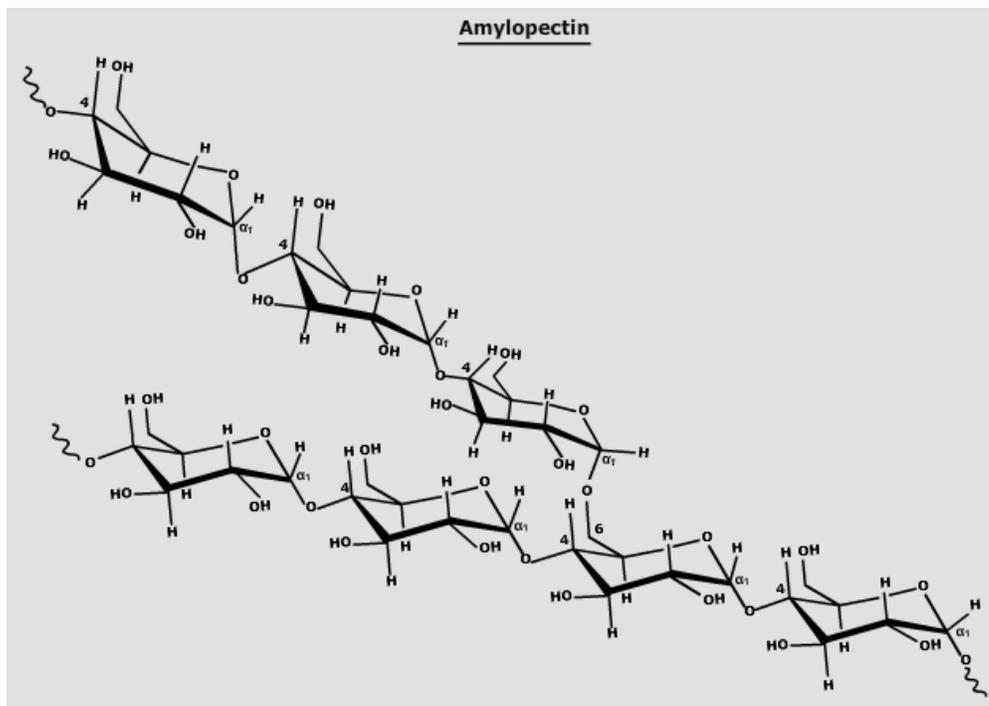
Fig-12.2: Amylopectin

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.
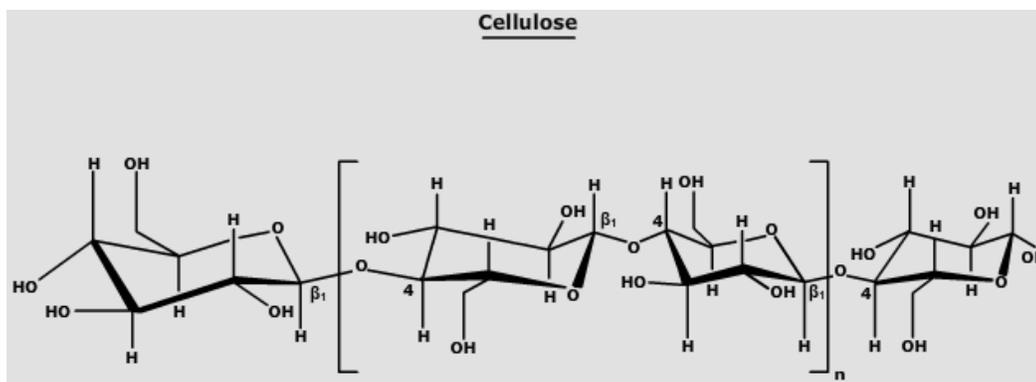
Fig-12.3: Cellulose
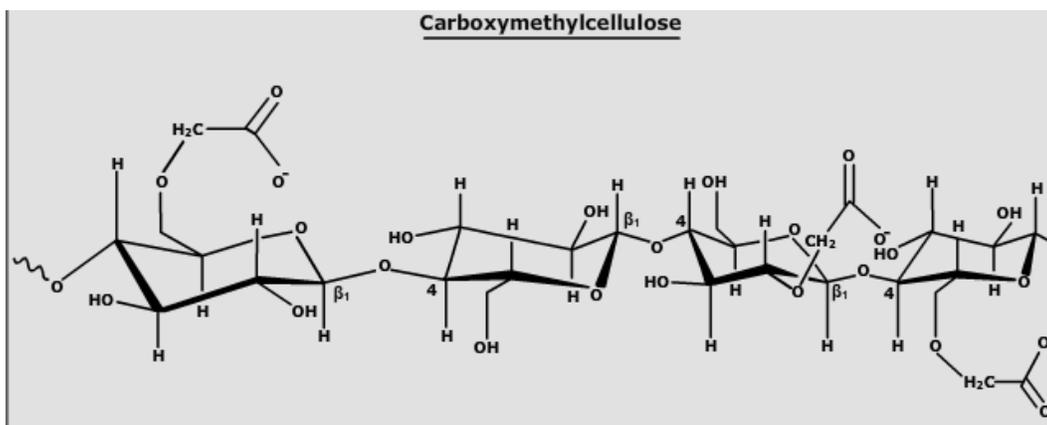
Fig-12.4: Carboxymethylcellulose

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 water-insoluble, 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.
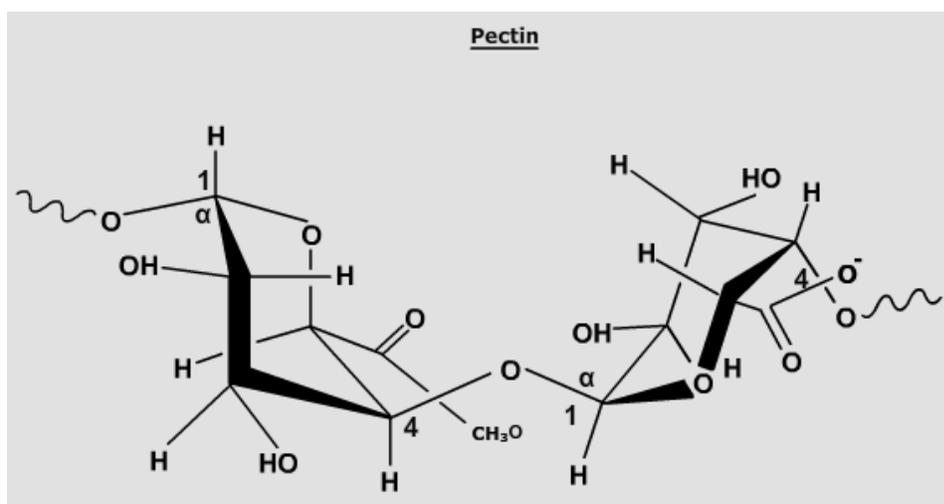
Fig-12.5: Pectin

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.
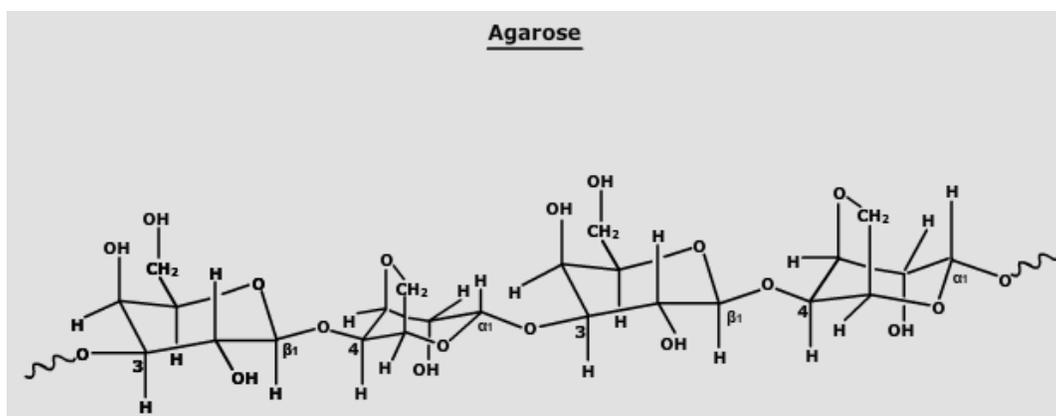
Fig-12.6: Agarose

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.
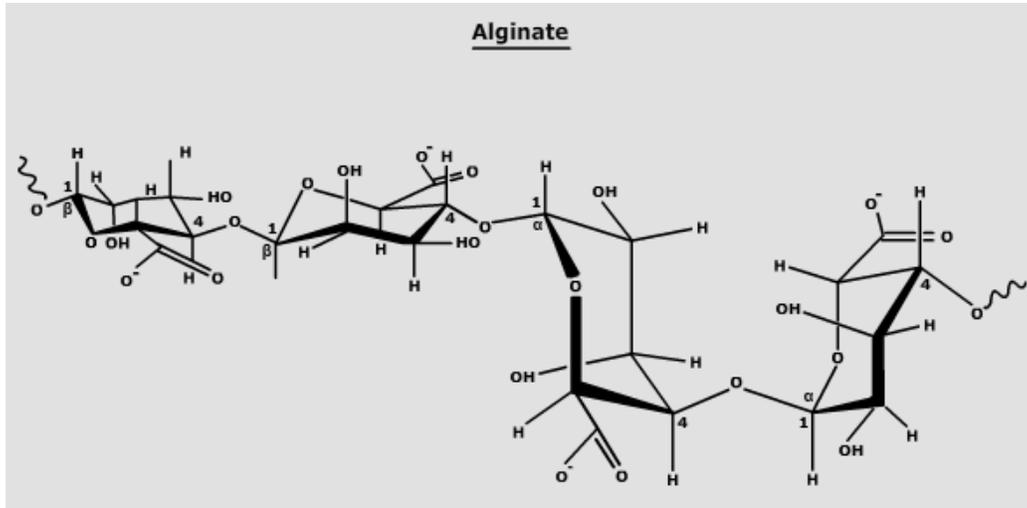
Fig-12.7: Alginate

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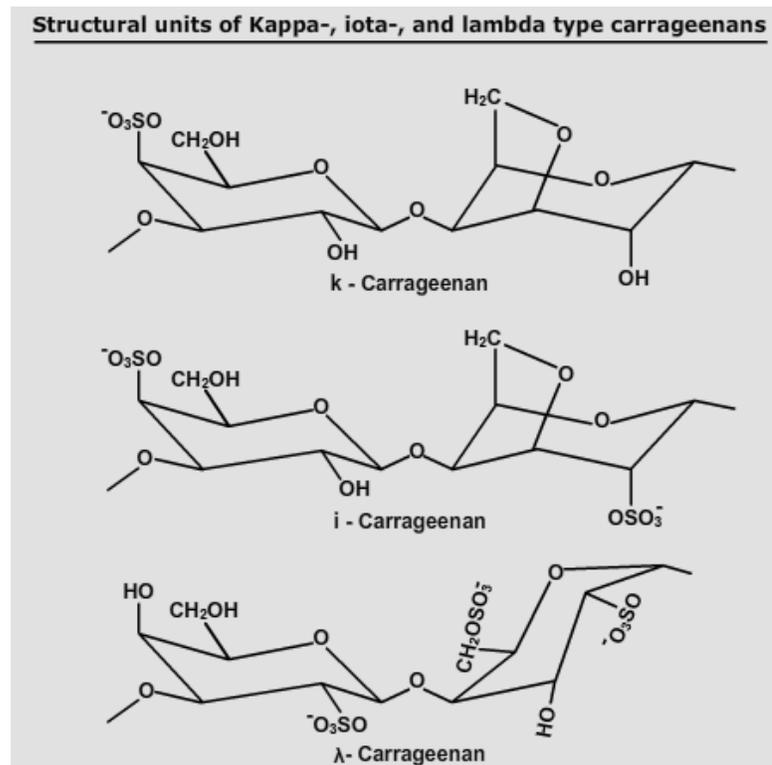
Fig-12.8: Structural units of kappa-, iota, and lambda type carrageenans

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° 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-D-mannose 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.
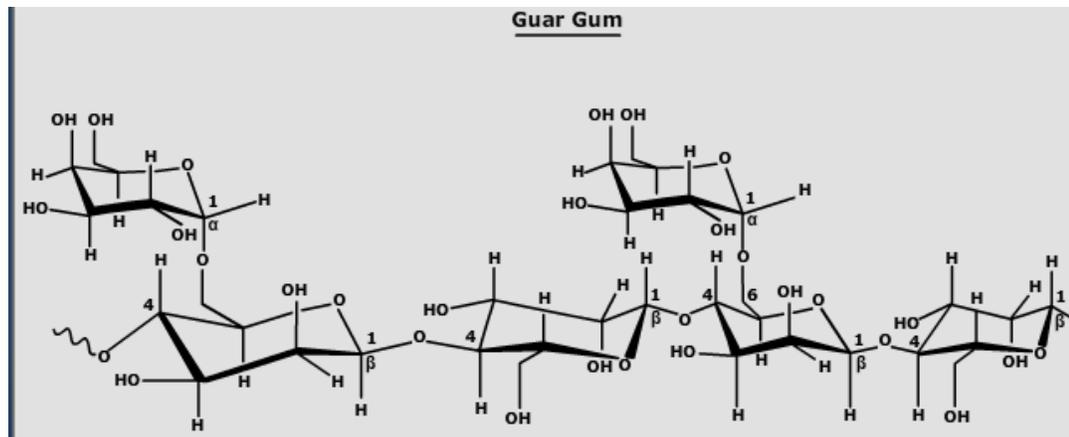
Fig-12.9: Guar Gum
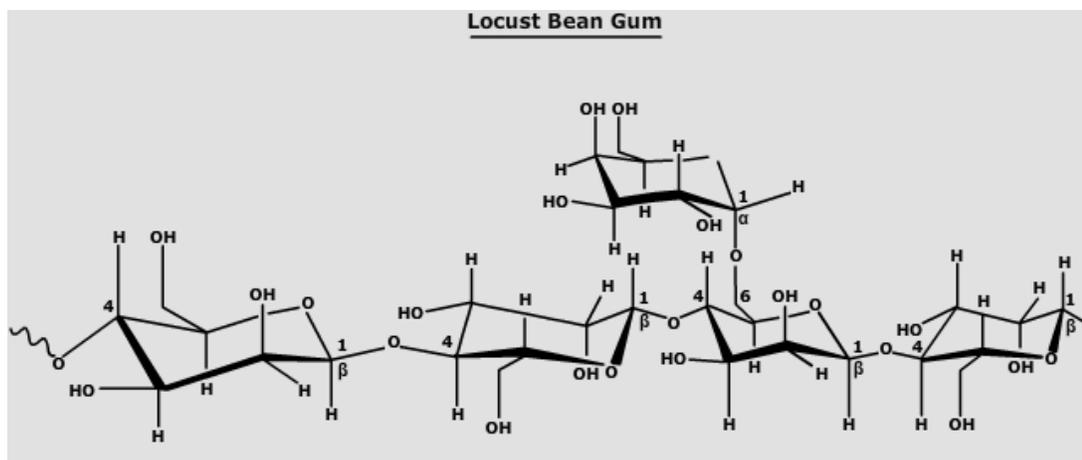
Fig-12.10: Locust Bean Gum

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Lesson-13

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.

Lesson-14
Main Elements and Trace Elements in Eggs, Cereals & Cereal
Products, Vegetables and Fruits

14.1. INTRODUCTION

While there is no universally accepted definition of "mineral" as it applies to food and nutrition, the term usually refers to elements other than C, H, O, and N that are present in foods. These four non mineral elements are present primarily in organic molecules and water, and constitute about 99% of the total number of atoms in living systems. Minerals are the constituents which remain as ash after the combustion of plant and animal tissues. Although mineral elements are present in relatively low concentrations in foods, they play key functional roles in foods. Mineral supply depends not only on the intake in food but primarily on the bioavailability, which is essentially related to the composition of the food. A series of food constituents, e. g., proteins, peptides, amino acids, polysaccharides, sugars, lignin, phytin, and organic acids, bind minerals and enhance or inhibit their absorption. They contribute to food flavor and activate or inhibit enzyme-catalyzed and other reactions, and they affect the texture of food.

14.2. CLASSIFICATION

Minerals have been classified as either major or trace, depending on their concentrations in plants and animals. The term "trace" was used to indicate the presence of an element that could not be measured accurately. Major minerals are calcium, phosphorus, magnesium, sodium, potassium, and chloride whereas trace elements include iron, iodine, zinc, selenium, chromium, copper, fluorine, lead, and tin. Minerals are divided into three classes:

MAIN ELEMENTS: The main elements (Na, K, Ca, Mg, Cl, P, S) are essential for human beings in amounts >50 mg/day.

TRACE ELEMENTS: Trace elements (Fe, I, F, Zn, Se, Cu, Mn, Cr, Mo, Co, Ni) are essential in concentrations of <50 mg/day.

ULTRA-TRACE ELEMENTS: Ultra-trace elements (Al, As, Ba, Bi, B, Br, Cd, Cs, Ge, Hg, Li, Pb, Rb, Sb, Si, Sm, Sn, Sr,Tl, Ti, W) are also very vital elements.

14.3. NUTRITIONAL AND FUNCTIONAL ROLES OF MINERALS

The important nutritional and physiological functions of the various minerals are discussed here.

14.3.1. MAIN ELEMENTS

Sodium: The sodium activates some enzymes, such as amylase. Sodium absorption is rapid. The excessive intake of sodium can lead to hypertension. A low intake of sodium can be achieved by a non-salty diet or by using diet salt (common salt substitutes).

Potassium: Potassium regulates the osmotic pressure within the cell, is involved in cell membrane transport. Potassium deficiency may be a result of undernourishment or predominant consumption of potassium-deficient foods, e. g., white bread, fat or oil. Potatoes and molasses are particularly abundant sources.

Magnesium: Magnesium is a constituent and activator of many enzymes, particularly those associated with the conversion of energy-rich phosphate compounds, and as a stabilizer of plasma membranes, intracellular membranes, and nucleic acids.

Calcium: It is abundant in the skeleton and in some body tissues. Calcium controls essential processes like muscle contraction (heartbeat), blood clotting, activity of brain cells and cell growth. The main source of calcium is milk and milk products, followed at a considerable distance by fruit and vegetables, cereal products, meat, fish and eggs.

Chloride: Chloride serves as a counter ion for sodium in extracellular fluid and for hydrogen ions in gastric juice. Chloride absorption is as rapid as its excretion in the urine.

Phosphorus: Phosphorus, in the form of phosphate, free or bound as an ester or present as an anhydride, plays an important role in metabolism and, as such, is an essential nutrient.

14.3.2. TRACE ELEMENTS

Iron: Most of it is present in the hemoglobin (blood) and myoglobin (muscle tissue) pigments. The metal is also present in a number of enzymes (peroxidase, catalase, hydroxylases and flavine enzymes), hence it is an essential ingredient of the daily diet. Two food processing problems arising from mineral fortification are the increased probability that oxidation will occur and, in the case of wheat flour, decreased baking quality. Generally, iron is an undesirable element in food processing; for example, iron catalyzes the oxidation of fat or oil, increases turbidity of wine and, as a constituent of drinking water, it supports the growth of iron-requiring bacteria.

Copper: Copper is a component of a number of oxidoreductase enzymes (cytochrome oxidase, superoxide dismutase, tyrosinase, uricase, amine oxidase). Copper is even less desirable than iron during food processing and storage since it catalyzes many unwanted reactions. Cu^{2+}-Ions are taste bearing. The threshold value 2.4–3.8mg/l was determined with aqueous solutions of $CuSO_4$ or $CuCl_2$.

Zinc: Zinc is a component of a number of enzymes (e. g., alcohol dehydrogenase, lactate dehydrogenase, malate dehydrogenase, glutamate dehydrogenase, carboxypeptidases A and B, and carbonic anhydrase). Other enzymes, e. g., dipeptidases, alkaline phosphatase, lecithinase and enolase, are activated by zinc and by some other divalent metal ions. Zinc deficiency in animals causes serious disorders, while high zinc intake by humans is toxic.

Manganese: Manganese is the metal activator for pyruvate carboxylase and, like some other divalent metal ions, it activates various enzymes, such as arginase, amino peptidase, alkaline phosphatase, lecithinase or enolase. Manganese, even in higher amounts, is relatively nontoxic.

Cobalt: Since it was discovered that vitamin B_{12} contains cobalt as its central atom, the nutritional importance of cobalt has been emphasized and it has been assigned the status of an essential element. Its requirement is met by normal nutrition.

Chromium: Chromium is important in the utilization of glucose. For instance, it activates the enzyme phosphoglucomutase and increases the activity of insulin; therefore, chromium deficiency causes a decrease in glucose tolerance.

Selenium: Depending on the region, it can vary greatly because of the varying content of selenium in the soil. Selenium is an antioxidant and can enhance tocopherol activity. The enzyme glutathione peroxidase contains selenium.

Molybdenum: It is a component of aldehyde oxidase and xanthine oxidase. The bacterial nitrate reductase involved in meat curing and pickling processes contains molybdenum.

Nickel: Nickel is an activator of a number of enzymes, e. g., alkaline phosphatase and oxalacetate decarboxylase, which can also be activated by other divalent metal ions. Nickel also enhances insulin activity.

Fluorine: The addition to drinking water of 0.5–1.5 ppm fluorine in the form of NaF or $(NH_4)_2SiF_6$ inhibits tooth decay. Its beneficial effect appears to be in retarding solubilization of tooth enamel and inhibiting the enzymes involved in development of caries. Toxic effects of fluorine appear at a level of 2 ppm. Therefore, the beneficial effects of fluoridating drinking water are disputed by some and it is a controversial topic of mineral nutrition.

Iodine: Iodine absorption from food occurs exclusively and rapidly as iodide and is utilized in the thyroid gland in the biosynthesis of the hormone thyroxine (tetraiodothyronine) and its less iodized form, triiodothyronine. Iodine deficiency results in enlargement of the thyroid gland (iodine-deficiency induced goiter). There is little iodine in most food. Good sources are milk, eggs and, above all, seafood. Drinking water contributes little to the body's iodine supply. To avoid diseases caused by low iodine supply, iodization of common salt is done in which potassium iodate, with 100 μg iodine added to 1–10 g NaCl. Higher amounts of iodine are toxic.

14.3.3. ULTRA-TRACE ELEMENTS

Tin: The natural level of tin in food is very low, but it can be increased in the case of foods canned in tinplate cans. Very acidic foods can often dissolve substantial amounts of tin. Thus, the concentration of tin in pineapple and grapefruit juice transported in poorly tin plated cans was 2 g∕l. The tin content of foods in tinplate cans is generally below 50mg∕kg and should not exceed 250 mg∕kg.

Aluminum: It is resorbed in only negligible amounts by the gastrointestinal tract. The largest portion is eliminated in feces. It is not secreted in milk.

Boron: Boron seems to be an essential nutrient, which promotes bone formation by interaction with calcium, magnesium and vitamin D. In addition, there are indications that boron is involved in the hydroxylation of steroids, e. g. in the synthesis testosterone. Important sources include wine and water.

Silicon: Silicon, as soluble silicic acid, is rapidly absorbed. The main source is cereal products. Silicon promotes growth and thus has a biological role. The toxicity of silicic acid is apparent only at concentrations ≥100 mg∕kg.

Arsenic: The main source is fish. Its metabolic role is not yet understood. It appears to be involved in the metabolism of methionine. Choline can be replaced by arsenocholine in some of its functions.

Table -14.1. Mineral content of eggs, cereals & cereal products, fruits and vegetable

Food products	Na	K	Ca	Fe	P
Eggs					
Chicken egg yolk	51	138	140	7.2	590
Chicken egg white	170	154	11	0.2	21
Cereals & cereal products					
Wheat, whole kernel	7.8	181	33	3.3	341
Wheat germ	5	993	49	8.5	1100
Corn flakes	915	120	13	2.0	59
Corn White	6	294	8	1.5	213
Rice, unpolished	10	238	16	3.2	282
Fruits					
Apple	1.2	122	5.8	0.25	12
Orange	1.4	165	42	0.19	23
Strawberry	1.4	161	21	0.64	29
Grapefruit	1.1	148	24	0.17	17
Plum	1.7	177	8.3	0.26	18
Vegetables					
Peas, green	2	274	24	1.7	113
Mushrooms	8	390	11	1.26	123
Potatoes	3.2	418	6.4	0.43	50
Carrots	60	321	37	0.39	35
Tomatoes	3.3	242	9.4	0.3	22

Table-14.2. Nutritional and Functional Roles of Minerals and Mineral Salts/Complexes in Foods

Mineral	Food sources	Function
Aluminum	Part of ant acids & leavening agents	Essential nutrient, Acts as leavening agent & Texture modifier
Bromine	Brominated flour	Improves baking quality of wheat flour.
Calcium	Dairy products, Green leafy vegetables	Deficiency leads to osteoporosis in later life. Texture modifier.
Copper	Meat, seafoods, nuts	Catalyst in lipid peroxidation, ascorbic acid oxidation.
Iodine	Iodized salt, seafood	Deficiency causes goiter. Improves baking quality of wheat flour.
Iron	Cereals, legumes, meat	Deficiency leads to anemia. Catalyze lipid peroxidation in foods.
Magnesium	Cereals, green leafy vegetables	Removal of Mg from chlorophyll changes color from green to brownish
Manganese	Grains, fruits, vegetables	Cofactor in enzymes like pyruvate carboxylase, superoxide dismutase.
Nickel	Plant foods	Widely used catalyst for hydrogenation of vegetable oils.
Phosphates	Animal products	Acidulent in soft drinks. Acts as a leavening acid. Helps in retention of moisture in meats. Phosphates are used for the emulsification of processed cheeses and meats.
Potassium	Fruits and Vegetables	KCl may be used as a salt substitute.
Selenium	Seafood, cereals	Cofactor of Glutathione peroxidase.
Sodium	NaCl, MSG, milk	Used as flavour modifier. Used as a preservative. Many sodium salts are used as leaving agents.
Sulfur	Widely available	Present in methionine and cystine - essential amino acids. Sulfur dioxide and sulfites inhibit both enzymatic and nonenzymatic browning. Prevents, controls microbial growth.
Zinc	Meats, cereals	ZnO is used in the lining of cans for proteinaceous foods to lessen formation of black FeS during heating. Zn can be added to green beans to help stabilize the color during canning.

$$*****\ \ddot\smile\ *****$$

Lesson 15. 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 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.

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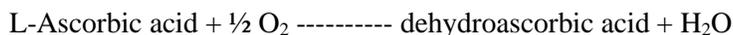
The reaction is significant in fruits and vegetables. It is responsible for the initiation of browning reaction, and for the eventual loss of all vitamin C activity.

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
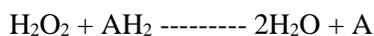
AH_2 is an oxidizable substrate.

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.

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Lesson 16.

Utilization in food industry and effect of inhibitors, pH and temperature

16.1. INTRODUCTION

A number of factors influence the rate of enzyme catalyzed reactions. The most important factors are Substrate concentration, Enzyme concentration, Temperature, pH, Specific activators, inhibitors. They are discussed below.

16.2. SUBSTRATE CONCENTRATION

For a given amount of enzyme under standard conditions, the initial reaction velocity varies with an increase of substrate concentration. At a low substrate concentration, the initial reaction velocity is nearly proportional to the substrate concentration (and the reaction is thus approximately first order with respect to the substrate). However, as the substrate concentration is increased, the initial rate falls off and is no longer approximately proportional to the substrate concentration (in this zone, the reaction is mixed order). With a a further increase in the substrate concentration, the reaction rate becomes essentially independent of substrate concentration and approaches a constant rate (in this range of substrate concentration the reaction is essentially zero order with respect to the substrate) and the enzyme is said to be saturated with substrate.
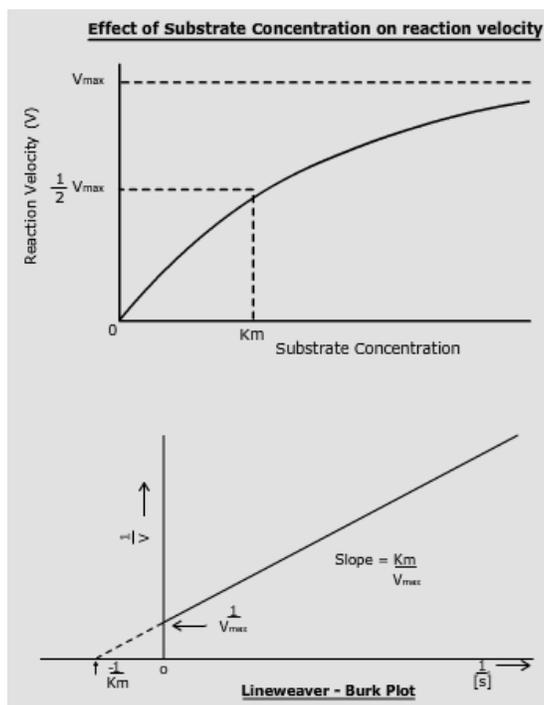
Fig-16.1: Effect of substrate concentration on reaction velocity

16.2.1. Michaelis-Menten Constant (K_m)

It is an equilibrium constant and is a measure of the affinity of an enzyme for its substrate. The more strongly an enzyme interacts with its substrate, the greater will be the proportion of the enzyme which is combined with substrate as ES, the lower the concentration of free enzyme, E and lower the value for K_m.

$$[E] + [S] \ll [ES] \circledR [E] + P$$

$$K_m = [E] [S] / [ES]$$

$K_m = [S]$, when $v_0 = \frac{1}{2} V_{max}$

16.2.2. Enzyme Concentration: For any enzyme, assuming the correct temperature and length of reaction time relationship, a medium at the optimum pH, and a constant substrate concentration, the curve shown in the Fig. is valid. If an excess of substrate is present, doubling the enzyme concentration usually doubles the rate of formation of end products. This usually applies at the start of the reaction, for the end products of the reaction often have an inhibitory effect on the enzyme, and decrease its efficiency. As the concentration of enzyme is increase, however, a point could (theoretically) be reached where the substrate (concentration held constant) is saturated with enzymes. If this point could be reached, further increases in enzyme concentration would have no influence on the rate of formation of end products.
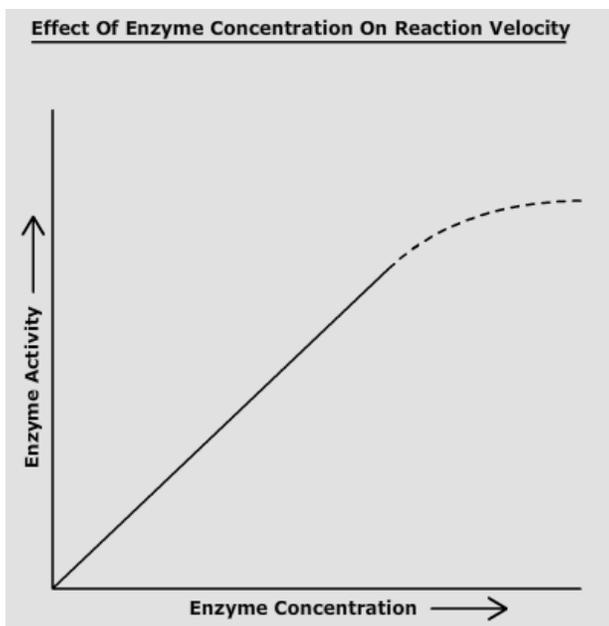
Fig-16.2: Effect of enzyme concentration on reaction velocity

16.2.3. Temperature: A curve of the type shown in Fig. is usually obtained if enzyme activity is related to variation in temperature. The rate of enzyme-catalyzed reaction at 0°C is close to zero. As the temperature is raised the reaction rate increases until a maximum is reached. At still higher temperature the rate decreases very

rapidly back toward zero. The temperature at which the maximum rate is observed is termed the optimum temperature. As the temperature increases, enzyme activity increases such that the rate of most enzymatic reactions approximately doubles for each 10°C rise in temperature, and is usually expressed as the temperature coefficient Q_{10}. The great majority of enzymes show optimal activity within the 30-40°C temperature range. At about 50°C, the enzyme becomes inactivated due to the denaturation of the apoenzyme, which results in the unfolding of the molecule and consequent loss of specifity.
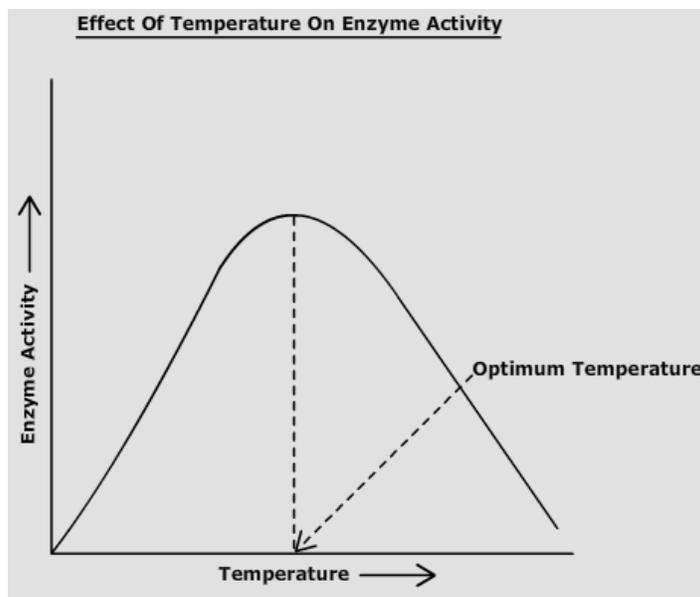
Fig-16.3: Effect of temperature on enzyme activity

The thermolability of enzymes is exploited to a high degree in the food industry. Pasteurization of milk involves exposure of milk to 63°C for 30 minutes. This treatment is sufficient to kill pathogenic bacteria such as *Mycobacterium tuberculosis*, and inactivates many enzymes. Effectiveness of pasteurization is determined by the absence of alkaline phosphatase activity. Blanching of fruits and vegetables is an essential pretreatment for fruits and vegetables for canning, freezing, and dehydration. This treatment is normally sufficient to inactivate all enzymes present. The effectiveness of blanching procedure can be determined by the absence of peroxidase activity.

16.2.4. pH effect : Enzymes are very sensitive to changes in the pH of their environment due to their proteinaceous nature. For every enzyme there is an optimum pH, which often lies within the range from 4.5 to 8.0, however, some few are most active in very acidic media, others in quite alkaline solutions. If enzyme activity is related to pH, the type curve shown in Fig. is obtained. Maximum activity is usually observed at or near their isoelectric point. Low catalytic activities are usually found in quite acidic or basic solutions. These effects are due in major degree to the gross denaturation of enzyme protein as well as change in the degree of ionization of functional groups of the enzyme involved in the active centre. Thus a pH change brings about conformation

changes in the protein structure, thus altering the active site of the enzyme for its steric fit with the substrate. If enzyme has more than one possible substrate, then the pH optimum can differ from each substrate.

16.2.5. Specific activators: Many kinases require Mg^{+2} ions, carbonic anhydrase requires, zinc, ascorbic oxidase requires copper, salivary amylase requires chloride for their full activities because they form co-ordination compounds and act as bridges between substrate and enzyme (proenzyme activity & coenzymes).

16.2.6. Inhibitors: Reversible Inhibitors : As the term implies the type of inhibition involves equilibrium between the enzyme and the inhibitors, the equilibrium constant (K_i) being a measure of the affinity of the inhibitor for the enzyme. Three distinct types of reversible inhibitors are known. 1. competitive, 2. noncompetitive, 3. uncompetitive.
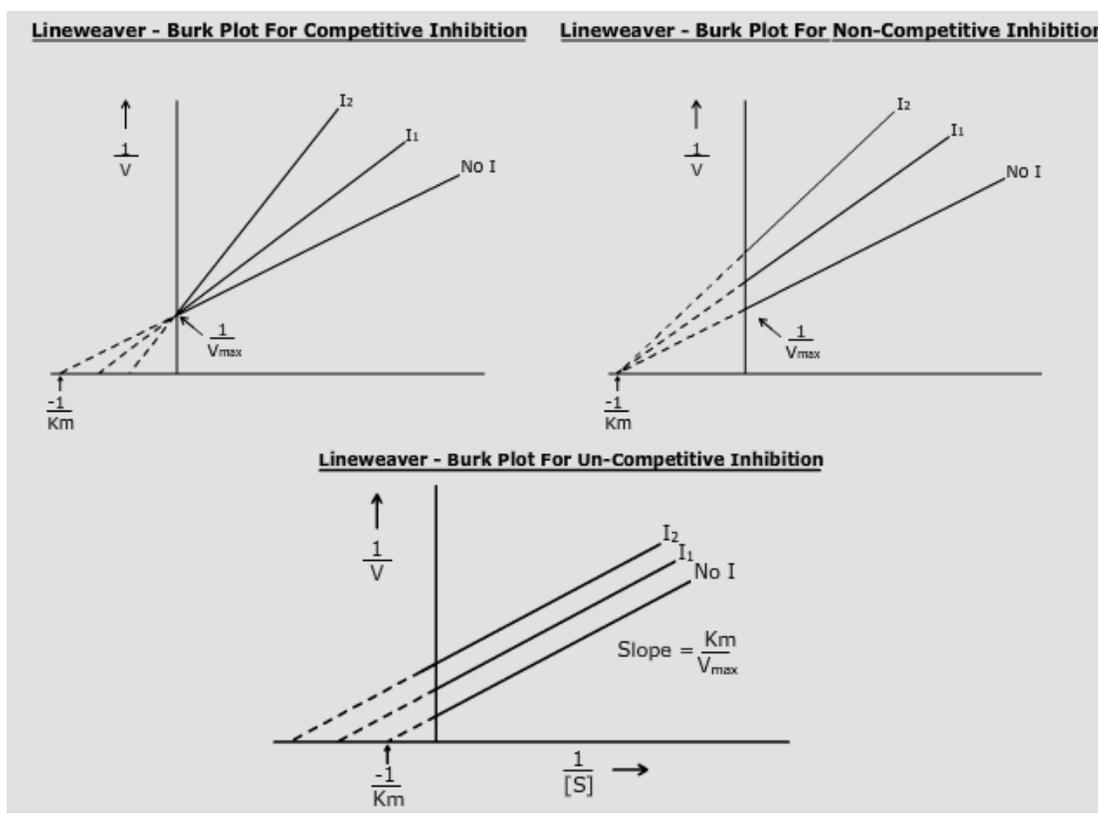
Fig-16.5: Effect of inhibitors on enzyme activity

a. Competitive: Compounds that may or may not be structurally related to the natural substrate combine reversibly with the enzyme at or near the active site. The inhibitor and the substrate therefore compete for the same site according to the above reaction. Succinic acid is the substrate of succinic acid dehydrogenase but its competitive inhibitors are malonic acid, oxalic acid, glutaric and phenyl propionic acid.

Note : Different Km values

No shift in V_{max}

b) Noncompetitive: Compounds that reversibly bind with either the enzyme of the enzyme-substrate complex are designated as non competitive inhibitors. This type of inhibition is not completely reversed by high substrate concentration since the closed sequence will occur regardless of the substrate concentration. Since the inhibitors binding site is not identical to nor does it modify the active site directly the K_m is not altered.

c) Uncompetitive Inhibition: Compounds that combine only with the ES complex but not with the free enzyme are called uncompetitive inhibitors. The inhibition is not overcome by high substrate concentrations, interestingly the K_m' value is consistently smaller than the K_m value of the uninhibited reaction, which implies the S is more effectively bound to the enzyme in the presence of the inhibitor. Uncompetitive inhibition is always a component of noncompetitive inhibition since in both cases EIS is formed.

16.3. Irreversible inhibitors

Forms a covalent bond with a specific function, usually an amino acid residue, which may, in some manner, be associated with the catalytic activity of the enzyme. In addition, there are many examples of enzyme inhibitors which covalently bind not at the active site, but physically block the active site. The inhibitor cannot be released by dilution or dialysis, kinetically, the concentration and hence the velocity of active enzyme is lowered in proportion to the concentration of the inhibitor and thus the effect is that of noncompetitive inhibition.

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Lesson 17

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 Food Additives

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.

Lesson .18

Aroma Compounds in Food & Flavour enhancers -monosodium glutamate, nucleotides

18.1. INTRODUCTION

When food is consumed, the interaction of taste, odour and texture provide an overall sensation, known as flavour". Flavour results from compounds that are classified in to two classes:

Those responsible for taste

Those responsible for odour

The latter are often designated as aroma substances. However, there are compounds, which provide both the sensations.

Compounds responsible for taste are generally non-volatile at room temperature. Therefore they interact only with taste receptors located in taste buds of tongue. Aroma substances are volatile compounds, which are perceived by the odour receptor sites of smell organ. i.e. the olfactory tissue of the nasal cavity.

Flavour enchancers are substances that enhance desirable flavours or depress undesirable flavours in foods.

18.2. CLASSIFICATION OF ODOURS

According to the site-fitting theory of odour perception, there are seven primary odours:

Camphoraceous

Ethereal

Musky

Floral

Minty

Pungent

Putrid

There are specific receptor sites for each of these above classes. The first three classes of odour perception depend primarily on the size of the molecule, the fourth and fifth classes on shape, the sixth on electrophillicity and the seventh on nucleophillicity.

A given odour is a mixture of appropriate primary odours e.g. almond aroma is a mixture of camphoraceous, floral and minty while garlic odour is mixture of pungent and putrid odours.

18.3. ODOUR THRESHOLD

The lowest concentration of a compound that is just enough for the recognition of its odour is called odour threshold value. When the detection threshold is lower i.e. the concentration at which the compound detectable for the aroma quality still can't be unambiguously established**.**

Compounds with high aroma value may contribute to the aroma of foods. The "aroma value" Ax of a compound is calculated according to the definition.

$$A_x = C_x / a_x$$

Here, C_x **is concentration of compound x in food.**

a_x is order threshold of compound x in food.

18.4. IMPACT COMPOUNDS OF NATURAL AROMA

The amount of volatile substances present in food is extremely low (10-15 mg/kg). In general, however, they compromise a large number of components, all of which are not important to food aroma. To be considered an aroma compound, the compound of volatile fraction must be present in a food in a concentration higher than its threshold value. A characteristic odour in a food can mainly be attributed to the combination of numerous volatile compounds, each of which smells differently. The difference in character of certain aroma partially due to varying proportions of these many widely distributed volatiles such as esters, acids, alcohols, aldehydes and ketones that occur in all foods.

These volatiles are called "contributory flavour compounds". However, most substances contain trace amounts of a few unique volatile compounds, which possess the characteristic essence of the odour. Such potentially important aroma compounds, which provide the characteristic aroma to the food, are "character impact compounds".

Based on the occurrence of such key compounds, foods can be divided into four groups:

(1) It includes those foods in which the aroma is decisively carried by one compound. The presence of other aroma compounds serves only to round off the characteristic aroma of the food. e.g. bananas-isopentyl acetate; Almond-benzaldehyde; Lemon-citral.

(2) Foods included in this group contain several aroma compounds, one of which may play a major role to create or determine the typical aroma of the food.

(3) The aroma of this group may be closely simulated or reproduced only with a large number of compounds. Usually character impact compound is not present e.g. processed foods like roasted coffee and some fruits like pineapple, peach, watermelon.

(4) The aroma of foods included in this group cannot be satisfactorily reproduced even with a large number of volatile compounds e.g. foods processed by fermentation like cocoa, beer and fruits like strawberry.

18.5. FLAVOUR ENHANCERS - MONOSODIUM GLUTAMATE, NUCLEOTIDES

Flavour enhancers have little or no favour of their own but small additions to a food product modify its flavour usually in desirable manner. An enhancer's effect is apparent to the senses as "feeling ", "volume", "body" or "freshness" (particularly in thermally processed foods) of the aroma, and also by the speed of the aroma perception.

18. 5.1. MONOSODIUM GLUTAMATE (MSG)

It has long been recognized as a flavour enhancer and is now been considered a primary taste, 'Umami' (derived from the Japanese for delightfulness).

The action of MSG was first observed by Japanese workers who were attempting to explain edible sea-weeds impart flavour enhancing properties to many foods. Although glutamic acid was firs isolated in 1866, the flavour enhancing properties of sodium were not discovered until 1909 by the Japanese chemist Ikeda. He found that MSG is the beneficial component of the algae L*aminaria japonica* used for a long time in Japan as a flavour improver of the soup and similarly prepared foods. The L- form the amino acid has the flavour enhancing property while the D-form is inert. MSG is now prepared from wheat gluten, beet sugar waste and soy protein.

Pure MSG is odorless. It is now generally agreed that glutamate flavour is unique. Pure MSG is detectable in concentrations as low as 0.03% and at 0.05% the taste is very strong. It is claimed that the compound intensifies the flavour of meat and vegetable through a rounding or blending effect. In addition glutamate is said to cause a "tingling" feeling of satisfaction or fullness. Apparently, glutamates stimulates our tactile sense as well as our taste receptors. The presence of salts is required to produce the glutamate effect. Glutamate taste is most effective in the pH range of 6-8 and decreases at a lower pH value.

MSG improves the flavour of many food products and is therefore widely used processed foods. Products benefited from the addition of glutamate include meat and poultry, soups, vegetables and sea foods. It does not have any effect on fruits or fruit juices or sweet spicy foods. It also suppresses undesirable flavour like sharpness of onion, rawness of many vegetables, earthiness of potatoes, bitterness in canned products of fish, meat, stews, soups etc.

The intake of large amounts of MSG by some hypersensitive persons can trigger a "Chinese restaurant syndrome". This is characterized by temporary disorder such as drowsiness, headache, stomach ache and

stiffening of joints. As a result, its use has been under scrutiny. It is argued that relatively high levels of MSG are naturally produced in certain foods such as well-aged cheese and tomato paste. Thus, the basic scientific question is why individuals who claim to experience adverse reactions to intentionally added MSG apparently do not experience similar reactions to naturally MSG.

18. 5. 2. 5'- nucleotides

The 5'- nucleotides especially 5'-insoniate and 5'-guanylate have enhancement properties similar to Mono Sodium Glutamate. Their flavour enhancing ability at 75-500 ppm is good in all foods. Sourness and sweetness are not affected. Additionally they improve the viscosity of liquid foods. There are three types of inosinic acid, 2'-,3'-,5,' – isomers has flavour activity. Both riboside 5'-phosphomonoester linkages are required for flavour activity. They also show a synergistic effect in the presence of glutamate.

18.5.3. OTHER

A different type of flavour enhancer is Maltol; (3-hydroxy-2-methyl-4-pyrone) has a caramel- like odor. It has the ability to enhance the perception of sweetness in carbohydrate rich food. e.g. fruit juices, marmalade, fruit jelly. It is useful as a flavour enhancer in chocolates, candies, ice cream, baked products, liquors and flavourings. It is used in concentration of 50-250 ppm. It is able to mask the bitter flavor of hops and cola. Addition of 5-75 ppm maltol allows a decrease of sugar content by about 15%, while retaining the sweetness intensity. Moreover maltol is reported to have antioxidant properties also. It has been found to prolong storage life of coffee and roasted cereal products.

Ethyl maltol [3-hydroxy-2-ethyl-4H-pyran-4-one] enhances the same aroma but is 4- to 6-times more powerful than maltol. It has not been detected as a natural constituent in food. Nevertheless, it is used for food aromatization.

$$*****\smiley*****$$

Lesson 19

Sugar substitutes-sorbitol,saccharin, cyclamate

19.1. INTRODUCTION

Sugar substitutes are those substances that are used like sugars forsweetening, but are metabolized without the influence of insulin. Importantsugar substitutes are sugar alcohols sorbitol, xylitol and mannitol and to acertain extent fructose.

19.3. CLASSIFICATION OF SWEETENERS

Sweeteners can be divided into two main groups: bulk and intensesweeteners:

Bulk sweeteners conferring body and texture to foods, are completelymetabolized by the body and provide an important part to our energy. They arealso referred to as nutritive or calorie sweeteners.

Intense sweeteners are generally not metabolized by the body, and areexcreted unchanged and used at very low levels in foods, hence are referred toas non- nutritive or non-calorie sweeteners. Unlike bulk sweeteners, these aregenerally not metabolized by the body and are excreted unchanged.

19.3.1. BULKSWEETENERS (NUTRITIVE)

Ø Sugars(refined sugars, sucrose, fructose, glucose, dextrose, maltose, etc.)

Ø Sugarreplacements/polyols/sugar alcohols (sorbitol, mannitol, xylitol, etc.)

19.3.2. INTENSE SWEETENERS (NON-NUTRITIVE)

Ø Natural ofplant origin (glycyrrhizin, steviosides, thaumatin)

Ø Synthetic(aspartame, acesulfame-K, saccharin, sucralose, cyclamate, etc.)

19.3.3. NUTRITIVE SWEETENERS

They are defined as the products that have greater than 2% of the calorific value of sucrose per equivalent unit of sweetening capacity. Typical of this food additive class is high fructose corn syrup. Not only it is sweeter than sucrose, it also adds body or thickness t liquid preparations. In addition it acts as humectants in semi moist food systems.

Polyols (known as sugar alcohols or polyhydric alcohols) are produced by hydrogenating corresponding reducing sugars. These nutritive sweeteners provide the bulk and texture of sucrose but can be labeled as fewer kcal /g. Members of this group include sorbitol(2.6 kcal/ gm), mannitol (1.6 kcal /gm), erythritol (0.2 kcal/g), lactitol (2kcal), iso malt (2kcal) and maltitol (3 kcal/g). Polyols do not provide browning in foods when baked. Polyols can combine with other alternative sweeteners for use in chewing gums, candies, frozen desserts and baked goods as well as in the development of new products, particularly those that offer neutraceutical benefits.

a) Sorbitol

Sorbitol, a polyalcohol, is approximately one half sweet ascompared to sucrose. It is hygroscopic in nature and also known as alcohol sugars. It is used as a sweetener for diabetics and in food canning. Sorbitol can be commercially prepared by catalytic hydrogenation of glucose. Acid-catalyzed elimination of water yields a mixture of 1,4-sorbitan (85%, I)and 3, 6-sorbitan (15%, II). Under more drastic conditions (action of concentrated acids), 1, 4:3, 6-dianhydrosorbitol (isosorbid III) is formed.

19.3.4. NON-NUTRITIVESWEETENERS

These include both naturally occurring and synthetic compounds that have elevated sweetness or sweetening power as compared to sucrose. As a result, they are usually incorporated at low levels into various foods as a replacement for sugar. These compounds make possible the manufacture of a wide range of low or reduced-calorie foods to serve the needs of people who have a need or desire to reduce their calorie intake. Although, the use of potentially useful non-nutritive sweeteners is growing, only a few are currently available for food application.

1. **Saccharin** : the primary non nutritive sweetener available currently is saccharin. Both the sodium and calcium salts of orthobenzo sulfimide are used. Sodium saccharin is the most commonly used because of its high solubility and stability. The level of use depends on the intensity of sweetness desired. In higher concentration, the compound has a light bitter after taste. It is about 300 times sweeter than sucrose in concentrations up tothe equivalent of a 10 % sucrose solution. The present stipulated ADI value is15 mg / kg of body weight.

2. **Cyclamates** : it is marketed as sodium or calcium salt of cyclo hexanesulfamic acid. The sweetening strength is substantially lower than of saccharin and is 30-40 times sweeter than sucrose. It has no bitter after taste. Over all, the sweet taste of cyclamate is not as pleasant as that of saccharin. Cyclamates were approved for use in US in 1950 but were later prohibited because of evidence suggesting they were carcinogenic. It has been approved in more than50 countries as a tabletop sweetener, and in sugar free beverages, baked good sand other low calorie foods. However, its use is banned by PFA.

3. **Sucralose** : it is high intensity, non caloric sweetener made from sugar. Sucralose is a free flowing, water-soluble white crystalline powder. It is a chlorinated sucrose derivative. It is said to be on average 600times sweeter than sucrose. It is formed by a process wherein three hydrogen-oxygen groups on the sugar molecule are replaced with three chlorine molecules. The small amount that is absorbed is not metabolized for energy. Itis also said to have excellent stability under a broad range of processing, pH and temperature conditions. It is also highly soluble in water and ethanol. Sucralose carries no health warnings. More than 100 studies have been conducted over 20 years to support safety claims, and no population subgroup has been excluded from using it. ADI is 5 g / kg body weight / day.

4. **Ace sulfame potassium (Ace-K)** : Ace-K, a non-caloric sweetener that is around 200 times sweeter than sucrose was approved by FDA for use innon alcoholic beverages. Ace-K is not metabolized, so it contributes nocalories. It has sweet, clean taste, and it remains stable under hightemperatures. Beverages containing it can be pasteurized under normalpasteurizing conditions with out loss of sweetness. One major advantage is itssynergy with other sweeteners, including nutritive and non-nutritive types.

19.4. CHARACTERISTICS OF AN IDEAL SWEETENER

An analysis of the organoleptic and functional properties of each single sweetener clearly shows that none of the currently known sugar substitutes comes close to the taste and functional properties of sucrose. Most exhibit one

or more differences like taste properties, e.g.: sweetness lag, lingering aftertaste or bitterness, lack of bulking properties, stability problems during storage and competitive prices. The characteristics of an ideal sweetener are:

Table-19.1: Characteristics of an ideal sweetener

Tastes like sugar	Very low or no calories
As sweet as or sweeter than sucrose	Tooth decay is prevented
Pleasant taste without any aftertaste	Useful in reduction of weight
Colorless and odorless	Diabetes management
High solubility	Non toxic
Stable in all processing conditions	Easily availability
Cost effective	

*****☺*****

Lesson 20

Food colours

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.

20.4. 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 (Lactoflavin ;

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 Carmoisine Erythrosine	16255 14720 45430	Azo Azo Xanthene
2.	Yellow	Tartrazine Sunset yellow FCF	19140 15985	Pyrazolone 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.

Module 8. Antinutritional factors and food contaminant

Lesson 21

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°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.

21.3. RADIONUCLIDES

Certain chemical elements are found in unstable states which spontaneously decay as they change to more stable forms. During this change they emit radioactivity as a-particles, b-particles or g-rays. Such radioactive atoms are called radionuclides. In living tissues they can lead to development of cancers or genetic abnormalities. (Table 21.1)

Table-21.1: Radionuclides

Emission	Nature
a particles	Two protons (positively charged) and two neutrons
β^- particles	Electrons (negatively charged)
γ rays	Electromagnetic radiation (no charge)
Neutrons	Subatomic particles (no charge)

21.3.1. Sources of Radionuclide Contamination:

1. **Naturally occurring radionuclides**: ^{40}K, ^{235}U, ^{232}Th, ^{14}C, ^{7}Be, ^{3}H, ^{87}Rb, ^{50}V, ^{115}In. The burning of coal and oil has been shown to lead to the distribution of such radionuclides as ^{40}K, ^{214}Bi, ^{214}Rb, ^{222}Rn and ^{226}Ra into the biosphere in the form of smoke and fly ash. Disseminated material is taken up from soil, incorporated into plant, eventually in animal products and finally in man.

2. **Testing of nuclear weapons**

3. Operation of reactors of all kinds and the application of atomic energy in medical, industrial and scientific uses.

The ground testing of fission or fusion of nuclear testing has resulted in widespread distribution of many different kinds of artificial radionuclides. Among these are the familiar ^{90}Sr, ^{89}Sr, ^{140}Ba, ^{137}Cs, ^{131}I, ^{140}La, ^{91}Ca and many, many other less familiar radioelements.

The actual steps utilized depend upon the mode of contamination, the chemical and physical characteristics of the radioelements, and the dietary patterns of the human population involved. Not all of these pathways will be involved in the dissemination of any one particular radionuclides.

In the case of ^{90}Sr the only pathway that if not of significance is that of direct atmosphere ® Man contamination. Strontium can be deposited on plants to be consumed directly by man, deposited on leaf surfaces to be consumed by animals whose products are then consumed by man, or deposited on soil and then be taken into plants to be consumed by man and animals. Radioiodine is an example of an abbreviated pathway brought about mainly by the short half-life of ^{131}I and most other iodines. The soil pathway is almost nonexistent because of the short half-life and so the major movement is from atmosphere to vegetation to the dairy cow to milk to man. The atmosphere ® man pathway can be of some significance for ^{131}I under special circumstances. While contaminant radionuclides can and do reach man through many different foods and pathways, milk is found to be a major contributor to the uptake of the fall out nuclides considered to be of most hazard to man, ^{90}Sr, ^{137}Cs, and ^{131}I. It is not that milk has a special propensity to accumulate harmful materials but that it is a product produced by animals whose food intakes represent large amounts of surface area which are susceptible to contamination, the handling of milk requires rapid movement to the consumer, and that milk constitutes a very important food item, especially for the very young.

Table-21.2: Radiological properties of some radionuclides

Radiological properties of some radionuclides			
Radionuclide	**Half-life**	**g-energy (MeV)***	**b-energy (MeV)***
I-131	8.05 days	0.36	0.61
Cs-137	30 years	0.66	0.52
Cs-134	2.06 years	0.79	0.66
Sr-90	29.1 years	-	0.55

* MeV = million electric volt

21.3.2. Some Radionuclides

21.3.2.1. Strontium-90: Strontium has half-life of 29 years and is b emitter. It is chemically similar to calcium and because of this, Sr-90 is found in greatest amounts in those foods, which are sources of Ca. A further consequence of this similarity is the fact that Sr whether Sr-90 or the natural non-radioactive variety is treated by the body in the same way as Ca and ultimately finds its way into the bone structure. Cows and other grazing animals can ingest Sr-90 as a result of eating contaminated grass. The Sr-90 becomes concentrated in these bones and also finds its way into cow's milk and hence into the human diet. In humans it is deposited in the bones and because of its radioactivity can cause bone tumors and leukemia. Children are very sensitive to this isotope because they require large amounts of Ca for bone formation and as a result deposit relatively more Sr-90. They also face a longer life span, which is important because radiation effects are cumulative.

21.3.2.2. Caesium-137: One of the alkali metals is chemically similar to sodium and potassium and its compounds are absorbed by the body in the same way as sodium compounds. Absorbed Cs-137 finds its way into the soft tissues of the body where it may cause genetic harm. Cs-137 is a g-emitter, half-life is 30 years. It is not retained in the body for a long time.

21.3.2.3. **Iodine-131**: It is a b-g-emitter, it accumulates in the thyroid gland. Iodine accumulates in the thyroid gland where it attains high concentration and radioisotope of I-131 is considered hazardous as it produces intense radiation, however, for short period after a serious fall out.

21.3.2.4. Carbon-14: is produced during the explosion of nuclear weapons and in the course of time it will be converted into CO_2 and may then be incorporated into plant tissues by photosynthesis. When plants are eaten by man or when animals which have eaten the plants are in turn eaten by man finds its way into any organ. C_{14}

decays very slowly and remains radioactive for thousands of years. Modern nuclear bombs are said to release into the atmosphere a much smaller amount of harmful radioactive isotopes than the original types. Nevertheless, contamination of the atmosphere still occurs when such bombs are exploded and this is why, apart from other more obvious reasons the testing of nuclear weapons should be avoided.

21.4. Toxic Trace Elements

Lead, mercury, cadmium, arsenic are the toxic trace metals of significance to man and are a "threat" to our foodstuffs by virtue of industrial usage.

21.4.1. Lead: Milk and milk products play an important part in man's diet and contribute greatly to the diet of the young, therefore, concern about the global pollution of the environment has led to many studies of the pathways of heavy metals into milk. Man has contributed significantly to the environmental pollution with lead which he has used in batteries, paints, alloys and as antiknock in petrol.

Milk itself does not come into contact with lead except possibly in canned milks where soldered joints historically caused problems. This was overcome by lacquering.

Fruit juices kept in glazed pottery can pick up lead because of acidic nature since most of the pottery is made with lead containing glazes.

The cow acts as a very effective biological filter diverting lead from her feed to her bones rather than to her milk. It has been shown that crops grown near busy highways have high lead contents, in some cases exceeding 100 ppm of lead in the dry matter. It has been estimated that nearly 90 per cent of the ingested lead is derived from food. However, only 5 per cent of this is absorbed.

Clinical Symptoms of lead toxicity include mild anemia, mental deterioration and aggressive behaviour, and kidney damage.

21.4.2. Mercury: Large amounts of mercury are released into the environment by several groups of industries. Major mercury users are the Chloralkali industry, where the mercury is used in electrolytic cells, the pulp and paper industry where mercury compounds are used as slimicides, and agriculture, where uses include seed dressings and sprays. Mercury is now known to be converted in sediments on river and lake bottoms into highly toxic methylmercury compounds.

Mercury is a cumulative poison and is stored mainly in the liver and kidney. Mercury in its pure metallic form is poorly absorbed, readily excreted from the body, and thus unlikely to cause poisoning. In contrast, the inorganic and organic compounds of mercury are highly toxic to humans.

Clinical Symptoms: Methylmercury accumulates in the human brain and thus neuro-toxic to both adults and the fetus. The clinical signs of methylmercury poisoning generally manifests in sensory disturbances in the

limbs, the tongue, and around the lips; irreversible damage to the central nervous system resulting in atoxia, tremor, slurred speech, tunnel vision blindness, loss of hearing, and death.

Minamata disease in Japan, named after the town of Minamata is due to mercury poisoning by consumption of mercury contaminated fish and shell fish obtained from water of Minamata bay. The causative agent, organic mercury compound is used in chemical industry as catalyst for the conversion of acetylene into acetaldehyde and vinyl chloride.

21.4.3. Cadmium: A significant part of man's intake results from inhalation from air contaminated by cadmium, and cigarette smokers increase their intake by 25-50%. Threat to milk comes from forage and fertilization of feedstuffs with sewage sludge. Cow acts as an effective biological filter and the proportion of ingested cadmium finding access to milk is extremely small.

Clinical Symptoms: Most of the absorbed cadmium is retained in the kidneys. Thus long term chronic ingestion of cadmium often results in serious renal damage, as well as bone disease leading to brittleness and even collapse of the skeleton. Abnormally high levels of cadmium in the diet also enhances the rates of several cancers in humans. Cadmium toxicity is the prime cause of Itai-Itai disease observed in certain population segments of Japan.

21.4.4. Arsenic:

Arsenic is usually classified as a metalloid since it has properties both of a metal and a non-metal. Arsenic trioxide and arsenic pentoxide are the two most toxic inorganic compounds. The major sources of arsenic are copper smelting and low grade coal combustion. Herbicides, burning of firewood and cowdung also contribute to environmental arsenic. Inorganic and organic arsenic compounds are absorbed in the gastrointestinal tract. The absorbed arsenic gets distributed via the blood stream to different organs. Chronic arsenic poisoning results in general muscular weakness, loss of appetite, nausea and inflammation of the mucous membranes of eyes, nose and lungs.

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Lesson 22

Individual constituents – proteins, lipids, carbohydrates and vitamins in cereals flour and their relationship in dough making

22.1. Introduction

Cereal products are amongst the most important staple foods of mankind. Nutrients provided by bread consumption in industrial countries meet close to 50% of the daily requirement of carbohydrates, one third of the proteins and 50–60% of vitamin B. Moreover, cereal products are also a source of minerals and trace elements. The major cereals are wheat, rye, rice, barley, millet and oats. Wheat and rye have a special role since only they are suitable for bread-making. Generally cereals belong to species of the wild grasses.

22.2 Structure of Cereal Grains

The main structural features of cereal grain are

1. **The embryo or germ:** The embryo is small and is attached to the base of the seed. It is the embryo from which the root and leaf of the new plant are formed. The germ is rich in lipids and high in total nitrogen and ash.

2. **Endosperm:** The endosperm makes up the major portion of the seed. It supplies the sprouting embryo with food in the period before the root and leaf begin to function. The endosperm cells consist of mainly of starch and proteins. The starch is form of spherical granules embedded in a matrix of protein. It is the main source of white flour and semolina.

3. **Bran**: It is dark coloured and consists of several layers of fibre. The layer of cells surrounding the endosperm is known as aleurone. In milling the aleurone layer separates with the bran. The bran has unusually high percent of crude fiber and ash. Crude fibre includes cellulose, hemicelluloses and lignins.

22.3 Composition of Cereal Grains

The composition of the cereals varies depending on variety, geographical and other conditions. The major compositional features are as follows:

Ø 80% of the dry matter of cereals is carbohydrates mainly starch and dietary fibers.

Ø Cereals generally contain 10-12% of protein. Proteins are found in all tissues of the cereal. Lysine content is low in all cereals. Methionine is also low, particularly in wheat, rice, barely and corn.

Ø Lipids are present to the extent of 1-2% in wheat and rice and 3% in maize, while oat contains as much as 5.7%. More lipids are present in germ and bran. Linoleic acid is the predominant fatty acid in cereal lipids.

Ø About 95% of the minerals are the phosphates and sulphates of potassium and magnesium. Cereals are poor sources of calcium and iron.

Ø Whole grain cereals are important sources of B vitamins. Since most of these vitamins are in the bran, refining or polishing the grains reduces vitamin B content.

Ø Cereals grains contain many enzymes of which amylases, proteases, lipases and oxido-reductases are of importance in cereal processing.

22.4. Individual constituents of wheat and their importance in baking

22.4.1. Proteins:

In 1907 T.B.Osborne separated wheat proteins on bases of solubility into four fractions.

Designation in wheat % Total wheat

Albumins: as leukosin in wheat 14.7

Globulins: as edestein. 7.0

Prolamins: as gliadin 32.6

Glutelins: as glutenin 45.7

Wheat flour contains soluble and insoluble protein fractions, the soluble proteins comprise 20% of total proteins and include albumins and globulins and certain minor glycoproteins. These proteins do not contribute to dough forming properties of wheat flour.

The insoluble wheat proteins are prolamins and glutenins that exist in ratio 2:3. They are also referred as gluten proteins. Gluten is the major storage protein in wheat. It is the heterogeneous mixture of gliadin and glutenin. Gluten is formed when water flour mixture is kneaded to form dough. Gluten proteins are responsible for formation of viscoelastic dough capable of entrapping gas during fermentation.

Both fractions of insoluble wheat proteins in hydrated forms have different effect on rheological characteristics of the dough. The prolamins are responsible mainly for viscosity of glutelins is responsible for dough elasticity. The gluten proteins in association with lipids are responsible for cohesive and viscoelastic flour properties of the dough that is suitable for making bread and other bakery products. Gluten consists of 90% of protein, 8% lipids and 2% carbohydrates.

22.4.1.1 Mechanism of Dough and Gluten Formation

Several physical and chemical transformations take place during mixing and kneading of the mixture of water and wheat flour. Under the applied shear and tensile forces, gluten proteins absorb water and partially unfold. The partial unfolding of proteins facilitates hydrophobic interactions and sulphydryl-disulfide

interchange reactions that result in formation of thread like polymers. These linear polymers in twin are believed to interact with each other presumably via hydrogen bonding, hydrophobic association and disulfide links to form a sheet like film capable of entrapping gas. Therefore optimum ratio of prolamins and glutelins is necessary to form a visco-elastic dough.

22.4.2. Lipids:

Cereals are generally considered to have low lipid content. Germ and bran of the grain contain higher concentration of fat than other parts. In wheat kernels-the germ and aleurone cells are rich in triglycerides, while phospholipids and glycolipids are predominant in endosperm. On an average, the wheat germ contains 6 to 11 % of the lipids, bran 3 to 5% and endosperm 0.8% to 1.5%. Wheat flour contains 1.5 to 2.5% lipids depending on milling extraction rate. Wheat flour lipids are differentiated by their solubility.

Non-starch lipids comprise of around 75% of total lipids of the flour. Remaining 25% lipids are bound to starch. The non-starch and starch bound lipids differ in their composition. In non-starch bound lipids the major constituents are triglycerides and glycolipids, while the starch bound lipids contains lysophosphatides. The non-starch lipids are further fractionated into free and bound form by solvent extraction. Both free and bound lipids contain non-polar glycolipids and phospholipid, the free lipids fractions contains 90% of the total non-polar lipids and 20% of total polar lipids of wheat flour.

By kneading the flour into dough the glycolipids becomes completely bound with gluten, while binding of other lipids is only 70 to 80 %, resulting in a starch-protein-lipid complex matrix. Non-starch lipids affect the rheological properties of dough. While starch bound lipids affect properties of the baked products.

The lipids are enclosed within the amylose helices, the lipids complexed within the starch granules retard swelling and increase their geletinization temperatures; thus they influence the baking behaviour of cereals and the properties of baked product. Polar lipids positively influence the gas holding capacity of the doughs and baking volume while non-polar lipids generally negatively influence the baking results.

22.4.3. Carbohydrates:

1) **Starch:** Starch is the major storage form of carbohydrate in cereals and occurs only in endosperm cells. Cereal starches consist of 25% amylose and 75% amylopectin. Starch granules swell when heated in water suspension. At the end of swelling they loose their native structural form and arrangement i.e. they are gelatinized.

    Starch contributes to viscoelastic strength of the semi rigid structure formed along with gluten, during baking Lipids and proteins are heterogeneous constituents of starch granules.

2) **Other polysaccharides**: Cereals contain polysaccharides other than starch, but in endosperm their content is less than that of starch. They include hemicelluloses, pentosans, cellulose, β-glucans and glucofructans.

These polysaccharides are primarily constitutents of cell wall and are more abundant in the outer portions of the kernels.

3) **Pentosans:** Its content in wheat flour is 2-3%. A portion of pentosans is water soluble. The soluble pentosans are able to absorb 25 times more water and thus can form highly viscous solution. The insoluble form of pentosans swells extensively in water. This portion increases crumb juiciness and chewablity of baked products. Pentosans play an important role in wheat baking quality since they also participate in gluten formation.

4) β-glucan: β-glucan content in wheat flour is only 0.5-2%. They are linear polysaccharides with D-glucopyranose units linked by β (1à3) and β (1à4) linkages. These polysaccharides are known as lichenins. They are slightly mucous and provide high viscosity to water solution.

Sugars: Sugars include mono-, di- and trisaccharides as well as other low molecular weight degradation products of starch. They occur in relatively low concentrations but are important for dough leavening in the presence of yeasts.

22.4.4. Enzymes: Cereal grains contain many enzymes and of these the amylases, proteinases, lipases and oxidoreductases play an important role in cereal processing. Some of them are described below.

1) **Amylases:** α- and β-amylases are found in all cereals. Their optimum activities are desirable in dough making in presence of yeast.

2) **Proteinases:** Acid Proteinases are present in wheat flour. It is possible that proteinases are involved in cleavage of gluten bonds, thereby affecting the softening or mellowing of gluten during baking.

3) **Lipases:** The lipases are responsible for the fatty acids appearing during the storage of cereals and their products.

Table- 22.1: Chemical Composition of Cereal Grains and Products

	Mositure	Protein	Fat	Carbohydrate	Crude Fibre	Minerals
Wheat (whole)	12.8	11.8	1.5	71.2	1.2	1.5
Wheat flour (whole)	12.2	12.1	1.7	69.4	1.9	2.7
Wheat flour (refined)	13.3	11.0	0.9	73.9	0.3	0.6
Wheat bread (brown)	39.0	8.8	1.4	49.0	1.2	-
Wheat bread (white)	39.0	7.8	0.7	51.9	0.7	-
Bajra	12.4	11.6	5.0	67.5	1.2	2.3
Barley	12.5	11.5	1.3	69.6	3.9	1.2
Jowar	11.9	10.4	1.9	72.6	1.6	1.6
Italian Millet	11.2	12.3	4.3	60.9	8.0	3.3
Maize	14.9	11.1	3.6	66.2	2.7	1.5
Ragi	13.1	7.3	1.3	72.0	3.6	2.7
Rice parboiled milled	13.3	6.4	0.4	79.0	0.2	0.7
Rice raw milled	13.7	6.8	0.5	78.2	0.2	0.6

(Source: Nutritive value of Indian Foods, National Institute of Nutrition (NIN), Indian Council of Medical Research (ICMR), Hyderabad, 1994)

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Lesson- 23

Type of flours for bread making and confectionaries and influence of additives

23.1. Introduction

The term baking is usually applied to flour-based foods, for example wheat, oats, maize and sorghum. These grains and flours have a relatively long shelf life. The main purpose of baking is to change the eating quality of the staple and to add variety to the diet. It is also a means of preservation, extending the shelf life by removing moisture and inhibiting enzymes and bacteria. After baking, goods such as breads and pastries have a shelf life of 2 to 5 days and other goods such as biscuits and some cakes have a shelf life of several months so long as they are correctly packaged. During baking food is heated by the hot air in the oven. Moisture at the surface is evaporated by the heat and a dry crust forms. Biscuit production involves slower heating so that moisture is also lost from the inside of the product.

Baked goods are produced from either doughs or batters which are a mixture of flour and water made by mixing, beating, kneading or folding. The processing method depends on the ingredients being used and the product being made. All baking is based around the use of wheat flour, but many other ingredients are also used, each of which has different effects on the final product. The commonest of these are listed here.

23.2. Flour

The word flour refers to the powder obtained from grinding a cereal grain. Flours from different wheat varieties vary in protein content. Flours that are good for bread making (i.e. give a good loaf volume) are obtained from wheat varieties which have high protein contents (12-14%). Good bread making wheat are described as 'hard'. Hardness is related to the degree of adhesion between starch and protein. Hardness and softness refer to the way in which the endosperm breaks down on milling. In hard wheat, fragmentation of the endosperm tends to occur along the lines of the cell boundaries, whereas the endosperm of soft wheat fractures in a random way. Hard wheat yields coarse, gritty flour and soft wheat give very fine flour. The strength of wheat relates to its baking quality. Hard wheat produces large loaf volume and good crumb structure. Dough of hard flour is more elastic and more resistant to stretching than the dough of soft flour. Bread flour should form good gluten when mixed with water resulting in bread with a good volume when baked. The flour of soft wheat is good for biscuits and cakes. These flours are usually obtained from soft wheat varieties. Their protein content is usually less than 10%. Biscuit flour should make dough having more extensibility, but less resistance than bread dough. They form only a small loaf with coarse crumb structure.

Characteristics of good cake flour

Cake flour is a medium-strength flour ground from soft low-protein wheat of low α-amylase activity and is very fine in structure. The purpose of flour in cakes is to allow an aerated structure to be retained after the cake has been built up. The stability of the final cake depends largely on the presence of uniformly swollen starch granules; hence, the starch granules should be undamaged during milling, be free from adherent protein, and be un attacked by amylolytic enzymes.

The best cakes are obtained from a low-protein flour (7-9%) which is soft and gives tender cakes. For cakes which contain a higher proportion of sugar than normal, the flour must be chlorinated. Good milling can help to achieve these characteristics, but obviously only if the wheat is already of the appropriate quality.

The characteristic and general quality of the flour depends on:

· The wheat variety and condition sunder which the wheat has grown.

- This affects the quality and quantity of gluten in the grain.

· The milling processes

- This determines the degree of separation of the bran and endosperm, as well as the particle size of the flour, an important factor in cake flour.

· Additives and special treatments used by the miller to produce flour mixes with special characteristics.

23.3. Yeast

The particular strain of yeast used in baked products is *Saccharomyces cervisiae*. After addition to the dough under appropriate conditions of pH 4-6 and temperature 30ºC, the yeast begins to feed on the starch in the mixture, forming sugar, alcohol and carbon dioxide. The bubbles of CO_2 cause the dough to expand ("rise"). The dough must be "kneaded" thoroughly to distribute the bubbles evenly and then left to rise again, usually to about double its original volume. If the mixture is left too long, acid produced by the oxidation of the alcohol causes the product to taste sour.

23.4. Chemical Leavening Agent

The most important chemical agent used in leavening is baking powder. Baking powder is essentially a mixture of $NaHCO_3$ and a weak solid acid or acid salt. When mixture dissolves in water and the temperature is raised, CO_2 is released according to this equation:

$$NaHCO_3 + H^+ \text{ (from the acid)} \rightarrow Na^+ + H_2O + CO_2$$
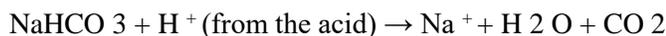
The most common acids used are potassium hydrogen tartrate, tartaric acid, acid calcium phosphate.

When baking powder is used rather than baking soda alone, the by-products are less alkaline

than Na_2CO_3 and thus they have no undesirable effects on the taste of the product. The type

of acid used in the baking powder affects the rate of CO_2 production, which in turn affects the

product. In baking, the rate at which CO_2 is produced and the continuity of CO_2 production are both important.

Baking powder is a very widely used ingredient in cooking and baking. Self-raising flour has also become popular in recent years. This is merely high-grade flour to which baking soda and a suitable acid (such as cream of tartar) have already been added,

Baking soda (sodium bicarbonate) is also used as leavening agent. It has the property of releasing CO_2 when it is heated:

$$2NaHCO_3 \rightarrow Na_2CO_3 + H_2O + CO_2$$

When used on its own, only half the available CO_2 is released and, more seriously, the sodium carbonate produced is strongly alkaline and gives the baked product a bitter, "soapy" taste and a yellow colour. During digestion of such products the Na_2CO_3 reacts with the HCl in the stomach to produce the other half of the available CO_2. For the above reasons, it must be stressed that sodium bicarbonate is very rarely used on its own, but generally mixed with some acidic material. To avoid an imbalance between the acidic and basic materials, i.e. an incorrect pH, baking powder is more commonly used.

23.5. Fat

Fat has a number of functions in baking. Fat weakens or 'shortens' a dough by weakening its gluten network, resulting in the baked product being softer, breaking easily and having a more tender mouthfeel. Fat can trap air during beating and mixing, producing a batter that consists of masses of tiny air bubbles trapped within droplets of fat. This is very important in cake baking in which it is these air bubbles that expand during baking forming a light, airy structure. In puff pastry fats which are soft over a wide temperature range are used. These can be spread between pastry layers and will separate them during cooking giving a layered pastry. Usually the fats used should have a bland flavour to prevent them from changing the flavour of the finished product, but occasionally fats are chosen on the basis of their flavour, e.g. using butter for particular baked goods and lard for meat pie pastry. In addition, the fat chosen needs to be able to form an emulsion with the other ingredients in the batter or dough.

23.6. Sugar

Sugar is most commonly thought of as a sweetener, but in baked goods it is also involved in

Several other processes. Sugar undergoes a series of complex browning reactions above 160°C and the products of these form the brown crust of many baked goods. The reactions are known as Maillard reactions and are essentially amino acid-catalysedcaramelisation reactions in which a sugar aldehyde or ketone is converted to an unsaturated aldehyde or ketone.

In addition, 0.5 - 0.75% w/w of sugar increases the rate of fermentation for fermented goods (i.e. breads) by giving the yeast more sugar to work on. In non-fermented goods such as biscuits, large quantities of sugar can be added. This improves the keeping quality of the biscuits as well as sweetening them.

23.7. Ascorbic acid (vitamin C)

Addition of a small amount (up to 100ppm) of vitamin C also shortens the time needed for the dough to mature. This is because ascorbic acid catalyses the gluten cross linking reactions to form a more extensible, elastic, strong network.

23.8. Egg

Eggs and their products constitute important ingredients in a wide range of bakery products. They improve the physical and organoleptic properties of the products in which they are used. Beaten egg white is used, like baking powder, to give the dough a light, airy texture. Eggs can be used as emulsifiers, moisteners (instead of simply adding water) and, nutritionally, as a source of fat and all the essential amino acids. Eggs improve the cell structure of the product, maintain it during the baking process, and reduce the moisture loss from the baked product.

23.9. Salt

Salt is added to enhance the flavour of cakes and breads and to "toughen up" the soft mixture

of fat and sugar. Salt has also a retarding effect on yeast fermentation. In its absence fermentation takes place very rapidly and the dough is too sticky to knead properly. The result is the coarse texture of the baked bread.

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Lesson 24

Physical, chemical changes during baking and determination of gluten and starch content in flour

24.1. Introduction

Several stages can be distinguished during the changes from dough to a baked product. They are as follows

--- Enzyme active stage (from 30°C to 70°C).

---Stage of starch gelatinization. (From 55 to 70°C)

---Stage of water evaporation

---Stage of browning and aroma formation.

These changes are different in the outer portion of the dough and in the interior of the crumb. This because in the oven since heat transfer occurs slowly in the dough, there is a steep temperature gradient inward from crust of the dough. The sequences of changes taking place during conversion of foamy texture of dough to spongy texture of bread and other product by baking at a temperature of 220°C to 250°C are as follows.

24.2 Chemical and physical changes

(1) When the dough is put in the oven, the rate of fermentation initially increases as heat is conducted through the dough. Upto 50°C, yeast produces CO_2 and ethanol at an increasing rate. At the same time the viscosity of dough falls rapidly and reaches to minimum at about 60°C. At the same time thermal expansion of gas within each cell result in rapid expansion of loaf volume, known as "oven spring".

(2) As the internal temperature of the dough increases above 37°C, activity of yeast decreases and gets inactivated at 54°C. At the same time, beyond 60°C, viscosity of dough again increases rapidly. This increase is caused by swelling of starch accompanied by release of amylose and also by protein denaturation. As the crumb starch gelatinizes at 65°C, the α and β-amylase present will attack the starch. The amylolytic activity continues until their enzymes are inactivated at about 74 °C. A optimum amylolytic activity is desirable to limit the degradation of gelatinized starch to counteract staling of bread.

At the same time the denatured protein, swollen and partially gelatinized starch forms a stable crumb network at about 74°C. This transformation continues until the end of baking when the internal temp reaches to 93 - 100°C. During this time gluten looses its tough and elastic state and becomes stiff and brittle.

This stiffens the starch structure so that a firm elastic crumb is formed. The starch granules of crust surface gelatinize almost completely. This is specially the case when "oven humidity" is high; the resultant starch film produces a pleasing glaze. This also retards drying and settling of the crust and permits full expansion of dough.

3) The above process results in tremendous increase in the tensile strength of the dough and the increase the presence of gas bubbles. Consequently the membrane gives way and becomes permeable, allowing H_2O, CO_2 & ethanol to evaporate. This results in baking weight loss. The internal temperature never exceeds 100 ºC but the outer temperature reaches nearly the oven temperature (~200 ºC). Thus water evaporates more from the surface & the crust is formed. This results in weight losses during crust formation upto 8-14% of the fresh dough weight.

At high temperature to which the outer part of the dough is exposed. Starch degrades to dextrin, mono and disaccharide at 110ºC-140ºC. Caramalization & non-enzymatic browning also occur at ~140-150 ºC providing the sweetness and colour to the crust. The roasted flavours developed at 150-200ºC.

In the crust heterocyclic compounds pyrroline and pyridine, as well as furanone and 2 and 3 methyl butanal are formed which are responsible for the roasty, malty and caramel flavour respectively in the products. The autoxidation products of linoleic acid, such as methional, and diacetyl are also involved in the aroma of the crumb.

24.3. Changes during storage Bread quality rapidly changes during storage. These changes are due to

1) Moisture adsorption- the crust loses its crispiness and glossiness.

2) The aroma compounds of freshly baked bread evaporates-resulting in loss of flavour.

3) Some of the very labile aroma compounds decrease rapidly on storage due to oxidation and other reactions.

4) The crumb structure also changes, although at a slower rate. The crumb becomes firm, its elasticity and juiciness are lost, and it crumbles easily. This is known as staling defect of crumb, which is basically a starch retrogradaton phenomenon.

24.4. Determination of gluten content in flour

General: Determination of the most important indices, which determine the loaf volume of bread, is the gluten content of the flour and an increase in the loaf volume of bread is noticed with an increase in the gluten content of the flour. Gluten exhibits the properties of cohesion, elasticity and viscosity which are the combined characteristics of its two insoluble component proteins i.e., glutenin and gliadin.

For good bread flour, wet gluten content ranges between 30 to 36 % and dry gluten content ranges between 10 to 12 %.

Apparatus:

1. Mortar

2. Glass rod

3. Hot air oven

4. Desiccator

5. Analytical balance

Principle: Gluten is separated out from the flour by washing the dough made using water. The albumins, globulins and other smaller proteins as well as starch are washed away with water leaving behind a cohesive, elastic and rubbery mass called crude wet gluten. The 65-75% of water present in crude wet gluten is dried out by drying at 100 °C for 24 h (or at 133 ± 2 °C for about 2 h) and weighed to get a value of dry gluten.

Procedure:

1. Weigh 25 g of flour and add about 15 ml of water and stir with a glass rod.

2. Mix it into a smooth and tight dough using fingers, taking care that handling loss is minimum and all the material is mixed into the dough.

3. Immerse the dough ball into water for about 1 h to ensure proper hydration.

4. Remove the dough ball and place it on a piece of blotting silk cloth having an aperture of 0.5 mm or 150 mm.

5. Wash it with a gentle stream of water till the water passing through the silk cloth does not contain starch i.e. the water does not turn blue when a drop of iodine solution is added (or the wash water is clear from turbidity of starch).

6. Collect the residue on the silk cloth and make it free of water by rubbing between dry palms or by using a suitable press.

7. Round it and weigh as wet gluten.

8. Dry this wet gluten in an oven maintained at 100 °C for 24 h (or break the wet gluten into pieces and dry in an oven maintained at 133 + 2 °C for 1-2 h).

Observations:

1. Weight of sample taken = W_1 g

2. Weight of wet gluten = W_2 g

3. Weight of dry gluten = W_3 g

Calculations:

A. % wet gluten = (W_2/W_1) x 100

B. % dry gluten = (W_3/W_1) x 100

24.5. Determination of starch content in flour

General: Starch is the major component of wheat flour. In wheat flour, starch granules are embedded in protein matrix. The major role of starch is to act as a water sink and set the system through partial gelatinization. Starch is also responsible for staling phenomenon since amylose fraction retrogrades rapidly during initial cooling of bread loaves. Slow changes in the amylo-pectin fraction are implicated in the further firming of bread during storage. Some of the normal starches get damaged during milling stage. Moderate amount of damaged starch is advisable while presence of excessive damaged starch is quite harm

Apparatus:

1. Conical Flasks

2. Funnel

3. Filter papers

4. Beakers

Reagents:

1. Fehling A and B solutions

2. Methylene blue indicator

3. Concentrated HCl

4. Standard glucose solution

5. 50% NaOH

6. Phenolphthalein indicator

Principle: The flour is suspended in water and undissociated residue containing starch is allowed to hydrolyse in the presence of dilute HCl. The glucose produced is filtered out and titrated against Fehling A and B using Lane-Eynon method. Value of glucose obtained is multiplied by 0.9 to get value of starch content present in flour.

Procedure:

1. Take 3 g flour sample in 50 ml cold water in a conical flask.

2. Stir it uniformly and keep it aside for 1 hr with occasional stirring.

3. Filter it and wash the residue with sufficient water.

4. Heat the undissolved residue for 2.5 hrs in 100 ml of 2.5% HCl solution in a flask equipped with a reflux condenser.

5. Cool, neutralize with NaOH and make up volume of 250 ml and filter it.

6. Fill the filtrate in burette.

7. Take 5 ml each of Fehling A and B solutions in a 250 ml conical flask. Add 20 ml of water and few pumice stones and bring to boil on burner.

8. Add into it sugar solution from burette until a faint blue colour remains.

9. Add 2-3 drops of methylene blue indicator and add sugar solution till red colour precipitates of Cu_2O is produced or obtained.

10. Record the volume sugar solution used for reduction of Fehling solutions.

11. Repeat the titration using standard glucose solution.

12. Calculate the sugar present in hydrolysate.

13. Convert the value of glucose to starch by multiplying with 0.9.

Observations:

1. Weight of sample taken = W gm

2. Final volume of starch hydrolysate = V_1 ml

3. Volume of standard glucose solution used = V_2 ml

4. Volume of starch hydrolysate used = V_3 ml

Calculations:

%Glucose = $\underline{V3 \times 2.5 \text{ mg glucose} \times V_1} \times 100$

$V_2 \times 1000 \times W$

%Starch = % glucose x 0.9

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Lesson 25. Classification, composition and physicochemical properties

25.1. Introduction

Legumes are an important food crop for both humans and other animals. They are generally low in fat and high in protein, and contain important micronutrients such as folate, potassium, iron and magnesium. Many food additives, such as gums for thickeners (e.g., gum arabic, guar gum and tragacanth gum), are derived from legumes. Soybean derivatives are used extensively in processed foods (such as soybean lecithin).

Many legumes are able to convert atmospheric nitrogen into a form that is usable by other plants. This is accomplished through a symbiotic relationship between the legume plant and special bacteria that live in nodules on the roots of the plant. Because of this ability to 'fix' nitrogen, legumes are essential for healthy ecosystems and agriculture. Grain legumes (pulses), complement cereals and make an ideal combination to provide protein quality matching that of animal products. Legumes contribute a major portion of lysine in the vegetarian diet. They are also fairly good sources of vitamins like thiamine, niacine and riboflavin and much needed iron, but relatively poor source of calcium and sulphur containing amino acids.

Legumes are classified in the family **Fabaceae,** which can be further divided into three sub-families – Faboideae, Mimosoideae and Caesalpinioideae. The sub-families (or families) are based on the form of the flowers, specifically the petal shape.

25.2. Structure

The grains of food legumes are similar in structure but differ significantly from each other in size, shape, colour and thickness of the seed coat. Legume seeds have two major parts; seed coat and the kernel (embryo and cotyledons). Pigeon pea, chickpea, black gram, green gram and horse gram have a seed coat accounting for 12-15 percent of the total weight of the grain where as it is in the range of 8-11 percent for lentils, French bean, kidney bean, pea, soybean and cowpea. On an average, pulses (including soybean) contain 11 percent seed coat, 2 percent embryo and 87 percent cotyledons. The embryo has two parts known as hypocotyl and plumule. Legume proteins are of two types – storage and structural– more versatile and useful in the Indian diets. Storage proteins (70-80 percent) occur within the cells in discrete protein bodies. About 20-30 percent are the structural proteins responsible for cellular activities including synthesis of structural and storage proteins. The cotyledons, account

for 93 percent of methionine and tryptophan of the whole seed, while the seed coat is the poorest in these amino acids. The embryo is rich in methionine and tryptophan but it contributes only about 2 percent of their total quantity in the seed. Legume proteins are deficient in methionine and trytophan Starch is the most abundant in legume carbohydrates. Legumes contain an appreciable amount of crude fiber. Cellulose and hemicellulose are the major constituents of crude fiber. Nearly 80-90 percent of crude fiber is present in the seed coat.

25.3. Chemical Composition

The chemical composition of food legumes vary and it is governed by the cultivar, geographical location and growth conditions. Legumes are rich in protein, carbohydrates and oil. They also contain good amount of dietary fiber and mineral. The grain legumes containing low oil (1-5 percent) and high protein (20-30 percent) and carbohydrates (50 percent and more) are called pulses and those having high oil (30-50 percent) and low protein (20-30 percent) are known as oilseeds in India. Soybean and groundnut are the two most cultivated legumes of the world.

25.3.1 Proteins: Proteins can be classified into three basic groups: globulins (70 percent), albumins (15 percent), and glutelins (15 percent). These protein fractions include essential and nonessential amino acids. All legume proteins have less than optimal content of sulphur amino acids, cystine and methionine and in some tryptophan is also deficient. Amino acid deficiency can be met by consuming large amount of legumes or by taking a mixture of legumes or by employing the complementary that exists between high sulphur amino acid cereals and legumes, especially the soybean. Cereals contain 7-14 percent protein whereas legumes have 20-40 percent. Lysine is the first nutritional limiting essential amino acid in most cereals, tryptophan is the second limiting amino acid in maize and the threonine in other cereals. Legumes are deficient in methionine and cysteine. In practice, cereals and legumes are eaten with other foods. The overall protein quality of cereal – legume mixtures is better than that of either protein source alone due to the complementary nature of their amino acid profiles. Digestibility of legume proteins is poor. However, it can be improved through heat-treatments like cooking, autoclaving, roasting, etc. The poor digestibility is due to the presence of protease inhibitors, deficiency of sulphur amino acid, presence of polyphenols and other anti-metabolites and tertiary structure of native proteins. It is important that this less-than optimal digestibility of legume be taken into consideration when one is attempting to meet nutritional requirements of humans with diets which are essentially legume-based. Legumes contain high protein content. It ranges between 17-25 %. Legume proteins are chiefly globulins. Albumins are also present in a few species. The quality of legume proteins is lower than most other classes of proteins. Although grain legumes are rich in protein their nutritive value is limited by the deficiency of sulphur containing amino acid. Low digestibility is another factor contributing to their poor nutritive value.

25.3.2. Carbohydrates: Legumes contains about 60 percent of carbohydrate. Starch is the principal carbohydrate. Minor amounts of lower molecular weight carbohydrates such as sucrose and sucrosyl oligosaccharides are present. The oligosaccharides include raffinose, stachyose and verbascose and they are associated with flatulence. It is the major hindrance to large-scale acceptance of legumes as food. Digestible energy coefficient for most legumes as well as mixed diets containing legumes are generally between 85-90 percent of the gross energy of the dry legume seed whereas metabolizable energy values are 75-85 percent. Soybean contains a considerable amount of carbohydrate such as galactans, pentoses, and hemicelluloses which are poorly utilized.

Legumes are good sources of dietary fibers. Low dietary fiber intake is linked with increased incidence of cancer of the colon and rectum, diverticular disease, coronary heart disease, diabetes and gallstone in affluent societies of the West. A concentrated source of dietary fiber from soybean is obtained by processing de-hulled and defatted soy flakes. It has 65-75 percent dietary fiber. Only 13 gm of soy fiber can provide 10 gm of dietary fiber in food whereas it takes 23 gm of wheat bran; 58 gm of oat bran; 502 gm of apple; and 735 gm of lettuce to provide 10 gm of fiber. The physiological benefits of soy fiber and other dietary fiber sources are increased fecal bulk and its moisture; reduced plasma cholesterol and positive influence on blood glucose and insulin concentration. The hypocholeslterolemic effect is attributed to the dietary fiber fraction of legumes because of its high content of pectins, gums and galactants. Dietary fiber also absorbs bile salt. It is aided by saponins.

25.3.3. Fat: The fat content of legumes is between 1 and 2 percent. Legume fats in general are rich in essential fatty acids.

25.3.4. Other nutrients: Most species of legumes contain only small amounts of provitamin A (50 to 300 International Units of vitamin A per 100g). Fresh legumes have more vitamin A activity. The thiamine content of legumes is approximately equivalent to that of whole cereals. Values range between 0.3 and 1.0 mg per 100 g. Legumes contain little riboflavin. Values range between from 0.1 to 0.4 mg per 100g. They are good source of niacin, containing on the average about 2.0 mg per 100 g. Dry legumes are almost devoid of ascorbic acid. The legumes are considerably richer in calcium than are most cereals. Average calcium content is 100 mg per 100 g. The legumes contain considerable amounts of phytic acid which may affect the absorption and utilization of their calcium. The legumes are good sources of iron. Values range between 2 to 10 mg per 100 g. Undecorticated legumes contain vitamin E in somewhat larger amounts than whole cereals. For pantothenic acid, the reverse relationship holds. Legumes are good sources of folic acid in most common foods.

25.3.5. Anti-nutritional Factors: Most of the legumes in raw form contain a wide variety of anti-nutritional factors or toxic principles like trypsin inhibitor and others. In addition, many of the grain legumes cause flatulence. Fortunately, most of the anti-nutritional factors are heat labile and are destroyed during cooking. These are chemical substances which, although non-toxic generate adverse physiological responses and interfere with

the utilization of nutrients. Anti-nutritional factors are protease inhibitors, lectins, goitrogens, antivitamins and phytates, saponins, oestrogens, flatulence factors, allergens and lysinoalanine. Some other anti-nutritional factors are cyanogens, favism factors, lathyrism factors, amylase inhibitors, tannins, aflatoxins and pressor amines. Although only a few legumes may contain all these anti-nutritional factors, many contain a few of them. Most of the anti-nutritional factors are heat labile and are destroyed during cooking. Heat stable compounds such as polyphenols and phytates are, however, not easily removed by simple soaking and heating. These could be reduced by germination and/or fermentation. Legumes are rich source of polyphenolic compounds. Till recently, some of these (e.g. tannins), were considered as anti-nutrients due to their adverse effects on protein digestibility. However, nowadays, there is considerable interest in the antioxidant activity of these compounds and in their potential health benefits, especially in the prevention of cancer and cardiovascular disease. Dark colored legumes like red kidney beans, black beans, black gram and soyabean have higher amount of these polyphenolic compounds.

Table No. 25.1 Chemical Composition Pulses and Legumes (g/100g of edible portion)

	Moisture	Protein	Fat	Carbohydrate	Crude Fibre	Minerals
Bengal Gram (whole)	9.8	17.1	5.3	60.9	3.9	3.0
Bengal Gram (dhal)	9.9	20.8	5.6	59.8	1.2	2.7
Black Gram (dhal)	10.9	24.0	1.4	59.6	0.9	3.2
Cow Pea	13.4	24.1	1.0	54.5	3.8	3.2
Field Bean (dry)	9.6	24.9	0.8	60.1	1.4	3.2
Green Gram (whole)	10.4	24.0	1.3	56.7	4.1	3.5
Green Gram (dhal)	10.1	24.5	1.2	59.9	0.8	3.5
Horse Gram (whole)	11.8	22.0	0.5	57.2	5.3	3.2
Khesari (dhal)	10.0	28.2	0.6	56.6	2.3	2.3
Lentil	12.4	25.1	0.7	59.0	0.7	2.1
Moth Beans	10.8	23.6	1.1	56.5	4.5	3.5
Peas (green)	72.9	7.2	0.1	15.9	4.0	0.8
Peas (dry)	16.0	19.7	1.1	56.5	4.5	2.2
Rajmah	12.0	22.9	1.3	60.6	4.8	3.2
Red Gram (dhal)	13.4	22.3	1.7	57.6	1.5	3.5
Soyabean	8.1	43.2	19.5	20.9	3.7	4.6

(Source: Nutritive value of Indian Foods, National Institute of Nutrition (NIN), Indian Council of Medical Research (ICMR), Hyderabad, 1994)

Lesson 26

Classification, general composition, chemical changes during ripening and storage

26.1. INTRODUCTION

Vegetables are defined as the fresh parts of plants which are consumed as such or cooked. More than ten thousand plant species are consumed as vegetables in the world. It provides essential nutrition to human in the form of minerals, dietary fibers, fats, vitamins, proteins, carbohydrates and energy. Fruits of perennial trees are not considered to be vegetables. Ripe seeds are also excluded (peas, beans, cereal grains, etc.).

Fruits include true fruits and spurious fruits, as well as seeds of cultivated and wild perennial plants. Fruits are commonly classified as pomaceous fruits, stone fruits, berries, tropical and subtropical fruits, hard-shelled dry fruits and wild fruits.

26.2. GENERAL CLASSIFICATION OF VEGETABLES

They are classified in following different groups with examples along with their consumption pattern.

1. **Mushrooms (edible species):** Fungi are commonly known as mushrooms. They are nutritious and provide characteristic flavour.
    1. e.g. Button, Flats, Truffle, Wood ear, etc. - Steamed, fried, dried, pickled or salted
2. **Algae (seaweed)**
    1. Sea lettuce - Eaten raw as a salad, cooked in soups
    2. Sweet tangle - Eaten raw in salads, cooked as a vegetable
3. **Rooty vegetables:** Usually a long or round-shaped taproot.
    1. Carrot - Eaten raw or cooked
    2. Radish – roots are eaten raw; used as a salad
    3. White (Irish) potato - Cooked, fried or deep fried in many forms
    4. Red beet, beetroot – Used as a salad
4. **Tuberous (rhizomatic) vegetables:** These grow underground on the root of a plant.
    1. Sweet potatoes - Cooked, fried or baked
    2. Cassava (manioc) - Cooked or roasted
5. **Bulbous rooty vegetables:** Usually grow just below the surface of the ground and produce a fleshy, leafy shoot above ground. Bulbs usually consist of layers or clustered segments. Only onions and garlic have prominent bulbs.
    1. Garlic – Consumed raw, cooked as seasoning
    2. Onion - Consumed raw, fried as seasoning, cooked as a vegetable
6. **Stem (shoot) vegetables:** The edible stalks of plants when the stalk is the main part of the vegetable.

a. Bamboo roots -Cooked for salads

1. **Leafy vegetables:** The edible leaves of plants.
    1. Cabbage - eaten raw in salads; cooked as a vegetable
    2. Spinach - Cooked as a vegetable

2. **Flower head vegetables:** The edible flowers of certain vegetables.
   1. Cauliflower - Cooked as a vegetable or used in salads
   2. Broccoli - cooked as a vegetable
3. **Seed vegetables:** It is also know as legumes. Seeds are usually obtained from pods. The pod is sometimes eaten along with the seed.
   1. Green beans - cooked as a vegetable, steamed or cooked for salads
   2. Green peas - cooked as a vegetable, steamed or pickled for salads
4. **Fruity vegetables:** Vegetable fruit are fleshy and contain seeds.
   1. Cucumber – Consumed raw in salads; cooked as a vegetable or pickled
   2. Capsicum - cooked as a vegetable
   3. Pumpkin - cooked as a vegetable
   4. Tomato - eaten raw, in salads, cooked as a vegetable, used as a paste or seasoned puree; immature green tomatoes are pickled and then eaten as salad

26.3. GENERAL COMPOSITION

The composition of vegetables can vary significantly depending on the cultivar and origin.

1. The amount of **dry matter** in most vegetables is between 10-20%. Some tubers and seed vegetables have high starch content and so a higher dry matter content.

2. Vegetables contain an average of 1-3% **N-compounds**. Of this 35-80% is protein and rest is amino acids, peptides and other compounds. The protein fraction consists to a great extent, of enzymes which may have either beneficial or detrimental effect on processing. They may contribute to typical flavour or to formation of undesirable flavour, tissue softening and discoloration. Enzymes of all the main groups are present in vegetables.

3. The **carbohydrate** content is in the range of 3-20%. The predominant sugars in vegetables are glucose, fructose and sucrose. Starch occurs widely as storage carbohydrate and is present in large amounts in some roots and tuber vegetables. Other polysaccharides viz. – cellulose, hemi-cellulose and pectinare there. The pectin fraction has a distinct role in tissue firmness of vegetables.

4. The **lipid** content of vegetables is generally low (0.1-0.9%). In addition to triacylglycerols, glycolipids and phospholipids are also present. Carotenoids are occasionally found in large amount.

5. The **organic acids** present in highest amounts in vegetables are malicand citric acids. The content of free titratable acids is 0.2-0.4 g/100 g fresh tissues. The pH is 5.5-6.5.

6. **Mineral** content of most vegetables is 1%. Potassium is the most abundant constituent followed by Ca, Na and Mg. The major anions are phosphates (PO_4^{2-}), chlorides (Cl^-) and carbonates (CO_3^{2-}).

7. Vitamins, flavouring compounds and dietary fibers are important **secondary constituents**. Plant pigments other than carotenoids and anthocyanins viz. chlorophyll and betalains are of great importance in vegetables.

26.4. STORAGE OF VEGETABLES

The storability of vegetables varies greatly and depends mostly on type, but also on vegetable quality. While some leafy vegetables, such as lettuce and spinach as well as beans, peas, cauliflower, cucumbers, asparagus and tomatoes have limited storage time, root and tuber vegetables, such as carrots, potatoes, kohlrabi, turnips, red table beets, celery, onions and late cabbage cultivars, can be stored for months. Cold

storage at high air humidity is the most appropriate. The relative air humidity has to be 80–95%. The weight loss experienced in these storage times is 2–10%. Ascorbic acid and carotene contents generally decrease with storage. Starch and protein degradation also occurs and there can be a rise in the free acid content of vegetables such as cauliflower, lettuce and spinach.

26.4. GENERAL CLASSIFICATION OF FRUITS

The most important fruits are classified as below along with their uses.

5. **Pomme fruits**
   1. Apple - Fresh, dried, purée, jelly, juice, apple cider, brandy
   2. Pear, - Fresh, dried, compote, brandy, Jelly
6. **Stone fruits**
   1. Apricot - Fresh, dried, compote, jam, juice, seed for persipan, brandy
   2. Peach - Fresh, compote, juice, brandy
7. **Berry fruits**
   1. Blackberry - Fresh, jam, jelly, juice, wine, liqueur
   2. Strawberry - Fresh, compote, jam, brandy
   3. Raspberry - Fresh, compote, jam, brandy
   4. Gooseberry - Unripe: compote; ripe: fresh, jam, juice
   5. Grapes - Fresh, dried (raisins) juice, wine, brandy
8. **Citrus fruits**
   1. Orange - Fresh, juice, marmelade
   2. Grapefruit - Fresh, juice
   3. Lemon – Juice
9. **Other tropical/ subtropical fruits**
   1. Pineapple – Fresh, compote, jam, juice
   2. Banana –Fresh, dried, cooked, baked
   3. Avocado – Fresh
   4. Date – Fresh, dried
   5. Guava – Compote, juice
   6. Mango – Fresh, compote, juice
   7. Watermelon – Fresh
   8. Papaya - Fresh, compote, juice
10. **Shell(nut) fruits**
    1. Cashew nut - Roasted
    2. Peanut – Roasted salted
    3. Almond - Baked and confectionary products
    4. Pistchio - Fresh, salted, sausage flavoring, decoration of baked products
    5. Wallnut - Fresh, baked and confectionary products, unripe fruits in vinegar and sugar-containing preserves
11. **Wild fruits**
    1. Rose hips - Jam, wine
    2. Sea buckthorn - Jam, juice
    3. Elderberry - Juice, jam

26.5. General COMPOSITION

Fruit composition can be strongly influenced by its variety and ripeness.

1. **Dry matter**: Dry matter content of most fruits (except nuts) varies between 10-20%. Major constituents are sugar, polysaccharides and organic acids, while Nitrogen-compounds and lipids are present in fewer amounts. Minor constituents include pigments and aroma substances of importance to organoleptic quality, and also vitamins and minerals of nutritional importance.

2. **Nitrogenous Compounds**: Fruits contain 0.1-1.5% Nitrogen-compounds of which 35-75% is protein. The protein fraction varies widely with fruit variety and ripeness. This fraction is primarily enzymes. Free amino acids are also widely distributed and are on an average 50% of the soluble N-content. A number of aliphatic &aromatic amines are found in various fruits.

3. **Sugars**: Glucose and fructose occur but in varying ratios in fruits. Sucrose is the dominant oligosaccharide. Some fruits like cherry, grape and figs have no sucrose. D-sorbitol is the sugar alcohol that is most abundant in pomme and stone fruits but absent in berries, citrus fruits, pineapple and banana. All fruits have cellulose, hemi-cellulose and pectins. Pectin fractions of fruits are especially affected by ripening. Starch is present mainly in unripe fruit sand generally its content decreases to a negligible level as ripening proceeds, with the exception of bananas.

4. **Lipid:** Lipid content of fruits is generally low (0.1-0.5%) with the exception of nuts. The fraction consists of TAGs, glyco- and phospholipids, carotenoids, **triterpenoids** and waxes. The presence of carotenoids is widespread in many fruits and in a number of fruits, viz. citrus fruits and peaches, their presence is the main factor determining the colour. The presence of carotenoids also forms the basis of fruit classification. The t**riterpenoid** fraction contains bitter compounds, viz. **Limonoids and cucurbitacins**. The fruit peel is often coated with a waxy layer.

5. **Organic Acids**: L-Malic acid and citric acid are the major organic acids in fruits. Malic acid is predominant in pomme and stone fruits, while citric acid is abundant in berries, citrus and tropical fruits. Tartaric acid occurs only in grapes.

6. **Phenolic compounds:** Phenolic compounds occur in most fruits and most of them contribute to colour and taste. They can form complexes during processing, resulting in dis-colouration of fruit pulp.

7. **Vitamins**: Many fruits are important sources of vitamin C; Pantothenic acid and biotin are present in some fruits, viz. Citrus fruits, figs, black currant; Vitamin B_{12}, Vitamin D and tocopherols are found in trace amounts.

8. **Minerals:** The most important cation is K^+, and the most important inorganic anionis PO^{2-}.

26.5. Physico-chemical changes during RIPENING OF FRUITS

It has been recognized for many years that fruits continue to undergo chemical changes after harvest until finally spoilage occurs. Ripening of fruits involves highly complex changes in chemical and physical properties. There are quite a number of intricate changes taking place in a complex bio-chemical system.

The most striking changes related to ripening are:

1. The cell-wall constituents are profoundly modified during ripening leading to softening.

2. The build-up of other cell constituents like starch and sugar are affected along with the disappearances of astringent compounds, which ultimately lead to increase in sweetness.

3. There is a formation of flavour and aroma compounds as well as changes in color due to breakdown of green chlorophyll pigment, whereby the yellowish color pigment of the shows up.

26.6. The changes TAKING PLACE ARE:

1. <u>Changes in respiration rate</u>

The respiration rate is affected by the development stage of fruits. A rise in respiration occurs with growth. This is followed by a slow decrease in respiration rate until the fruit is fully ripe. In a number of fruits, ripening is associated with a renewed rise in respiration rate soon after picking, until it reaches a climax, which is known as **climacteric respiration**. This increase in respiration is referred to as **climactericrise**. This is followed by a steady decrease in respiration rate i.e. senescence. Depending on the fruit, this can occur before/after harvesting Maximum CO_2 production occurs in climacteric stage.

The climacteric rise is so specific that fruits can be classified into:

      a) **Climacteric fruits**: apples, banana, pears, mango, papaya, tomato

      b) **Non-climacteric fruits**: pineapple, oranges, strawberry, grapes and lemon.

      It should be emphasized that **non-climacteric fruits generally ripen on plants and contain no starch.**

2. <u>Changes in metabolic pathways</u>:

Metabolic shift may occur in several fruits during ripening e.g. during ripening of banana, it appears that the Embden-Meyerh off Pathway (Glycolysis) becomes dominant and Pentose Phosphate Pathway is suppressed during ripening.

3. <u>Change in individual constituents offruits</u> :

Carbohydrates: During ripening, significant changes occur in the carbohydrate fraction. In green fruits, usually have abundant starch and less soluble sugars. On ripening, starch content decreases, while sugar content increases, which gives the ripe fruit its sweetness. It has been assumed that sugars are produced at the expense of starch. It also appears that in addition to starch, other sugars are also available for conversion. A decrease in hemi-cellulose content observed during the ripening of banana suggests that they can be a possible source of sugars. Additionally, organic acids may also be a possible source.

Another obvious change in the fruits is the alteration of texture. The softening of the fruit tissue, during ripening is associated with remarkable changes in pectin fraction. Insoluble protopectin is increasingly transformed into soluble forms. Protopectin is tightly associated with the cellulose in the cell wall matrix. So, this conversion can decreaserigidity of the matrix. Additionally a decrease in the degree of methylation of pectin (from ~80% to ~40%) and the decrease in the degree of polymerization of pectins have also been observed in fruits, viz. bananas, citrus fruits, mango,melons, etc. All these together contribute to an increase in softness of the ripe fruit. Moreover, the soluble pectins bind the polyphenols and there by quench their astringent effect and thus contribute to the mild taste of ripe fruits.

Proteins: During ripening of some fruits, although the total nitrogen content is constant, an increase in the protein content is observed, which is mainly due to biosynthesis of enzymes. During ripening, a shift also occurs in the amino acid and the amine fraction. These shifts are, however, not uniform and are affected by type and ripening stage of fruits.

Lipids: Little is known about changes in lipids. Changes have been found in the composition and quantity of lipids, esp. in phospholipid fraction.

Acids: There is a decrease in the acid content of fruits during the ripening with the exception of lemon. There can be the changes in the proportion of various acids. e.g. in ripe apples, malic acid is the major acid, while in unripe ones, quinnic acid is the major one. In many fruits, synthesis of ascorbic acid takes place during ripening.

Pigments: Ripening of fruits is usually accompanied by a decrease in colour. The transformation from green to other colour is due to the degradation of chlorophyll and the consequent appearance of the concealed pigments. In some fruits, the change is more due to synthesis of other pigments, e.g. lycopene content of tomatoes greatly increases during ripening.

Aroma compounds: Formation of typical aroma compounds occurs during ripening. In bananas, for example, noticeable amounts of volatile compounds are formed 24hrs after climacteric stage has passed. Aroma buildup is affected by external factors, viz. temperature and day-night variations.

Water: Living parts constantly transfer H_2O to the surroundings. This loss of water gradually results in visible shriveling. This is especially because when fruit is plucked, H_2O flow into fruits is discontinued, even though H_2O loss continues. This loss is high at high temperature and dry atmosphere. This H_2O given off through physiological forces that remain active even after harvesting is called **transpiration**. Water is also formed due to **respiration**. Most of this water formed is removed through evaporation along with readily accessible surface water. However, some fruit shave a waxy layer on the skin to check the loss of H_2O.

$$***** \ \ddot\smile \ *****$$

Module 12. Jams, Jellies and Pickles

Lesson 27

Classification, composition and preservation

27.1. INTRODUCTION

Jams and jellies are products made principally from fruits, but they can also be made from some vegetable materials, such as sweet potatoes, tomatoes, carrots, and some legumes. Generally a preserve or jam is a product manufactured with one or a permitted combination of fruit ingredients, and one or any combination of some optional ingredients. Fruit ingredients should be mature and properly prepared, including fresh, concentrated, frozen, or canned. Pickling is an ancient art of food preservation. It is the process of preserving food articles by anaerobic fermentation in brine (salt solution) to produce acid, or storing it in acid solution (usually vinegar). The resulting food preparation is called a pickle, i.e edible product preserved and flavoured in a solution of common salt and vinegar along with spices and oils.

27.2. JAMS AND JELLIES

Jams are produced usually from one kind of fruit. They are thickened by boiling and constant stirring of the whole or sliced fresh or fresh stored raw material, or of fruit pulp. Ordinary jams are also made from fruit slurry. Boiling under a vacuum at 65–80 ℃ offers the advantage of preserving the aroma and color. The disadvantages are the absence of sucrose inversion and the low caramalization. These reactions produce the characteristic taste of jams boiled in an open kettle. The optimal pH of 3.0 required for gelling is adjusted by the addition of lactic, citric or tartaric acid, if necessary.

Jellies are gelled food made of one or a permitted combination of fruit juice ingredients and one or any combination of the optional ingredients. Such a mixture is concentrated with or without heat. Fruit juice used in jelly manufacture is the filtered or strained liquid extracted with or without application of heat and with or without addition of water from mature, properly prepared fruits that are fresh, frozen, or canned. Some of the fruits used for making fruit jellies include apple, apricot, blackberry (other than dewberry), black raspberry, cherry, fig, gooseberry, grape, grapefruit, guava, orange, peach, pineapple, pomegranate, pear, quince, raspberry, red raspberry, strawberry.

27.2.1. ESSENTIAL INGREDIENTS IN JAMS AND JELLIES

Jams and jellies are products based in texture formation. They are characterized by the formation of a special viscous structure in jams and gel formation in jellies, and both properties are developed by the interaction of sugar, pectic substances, and acidity (pH). In jams, the viscosity is the result of an interaction between sugar and

pectin in the presence of high fiber content. All cell wall materials are present in the product and the effects of cellulose and hemicellulose molecules do not permit the formation of a continuous gel. In jellies, clarified or strained juices with very low fiber content are used; hence the relationship between pectin and sugar permits the formation of a continuous gel structure.

a) **Sugar:** Most jams and jellies are added with 65% of sugar. The types and concentrations of sugar are responsible for some of the taste in jams and jellies; added sugar, normally sucrose, does not have the same effect on this important quality factor. Jam and jellies fall under the category of so-called intermediate products, having a_w of 0.80 to 0.85. These products are not self-preserved because the water activity values are not low enough to control microbial growth or chemical reactions. The principal microbiological problems are molds and yeast, not bacteria.

b) **Pectin:** Pectin is a very complex molecule formed by a polymer of D-galacturonic acid. The degree of esterification indicates the capacity of the pectin to form a gel. Gel formation is produced by the relationship between pectin, water in the fruit, and sugar, under a controlled pH. High methoxyl pectins gel at acid pH (less than 3.5) in the presence of sugar. Low methoxyl pectins, on the other hand, gel at higher pH in the presence of some divalent cations, of which the most relevant is Ca^{2+}.
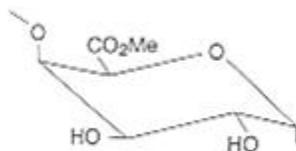
Figure: Monomeric unit of high-methoxyl pectin.

c) **Acid:** Acid is also an essential component in jams and jellies. Normally fruit used for making jams and jellies has a low pH. Acid stabilizes the relation between pectin and sugar. Berries have low pH due to their content of some common organic acids, such as ascorbic, citric, tartaric, and malic acid. All these acids can be used to increase the acidity in jams and jellies. Acids also help to produce the inversion of sugar at the beginning of the process. Sucrose is converted into glucose and fructose, which may improve the quality of products by increasing the brightness, reducing crystallization, and reducing the sugar flavor in products.

Table- 27.1: Composition of various jams (average values in %)

Fruits Jam	Moisture	Total sugar	Total acid	Ash	Dietary fiber
Strawberries	35.0	58.7	0.89	0.23	0.80
Apricots	36.9	51.3	1.14	0.28	0.60
Cherries	36.6	57.3	1.26	0.28	0.50
Blackberries	34.2	58.0	0.37	0.24	1.20
Raspberries	35.9	54.6	1.03	0.23	1.20
Plums/prunes	31.1	59.1	0.42	0.24	0.43

27.2.2. Mechanism of formation of pectin gels

The most commonly accepted theory of gel formation is that the mechanism of jellying involves stacking of polysaccharide chains to form junction zones. For gel formation sugar, acid, water, pectin must be present. Protons of the acid shift the equilibrium between ionized and unionized groups. Added sugar further decreases the hydration of pectin by competing for water, there by lowering a_w. Hence water is less free to solvate the polysaccharide and so there is increased hydrophobic interaction between methyl esters group. Thus, due to loss of some of their charges and hydration, the polymer molecules can now associate over a portion of their length forming junctions and thus a network of polymer chain is formed that entraps aqueous solution of solute molecules. Finally when cooled the unstable dispersion of hydrated pectin forms gels.

27.2.3. Factors affecting gel formation

A firm gel is that which is firm enough to stand without appreciable deformation and yet tender enough to spread readily on bread. A firm gel depends on following factors:

a) Pectin: As % pectin increase in the mixture, the firmness of jellies produced on cooling increases. A satisfactory jelly is obtained with around 1% pectin, but quantity will vary with quality of pectin preparation, average molecular weight of pectin molecules and degree of methylation.

b) Degree of methylation in pectin: Excellent jellies can be prepared from pectin with wide range of methoxy content but max jellying appears at about 8%. This represents esterification of half of the carboxyl group. By definition, preparations in which more than ½ of the carboxyl groups are in methyl ester forms are classified as high methoxy pectins. The high methoxy pectines gel when sufficient acid and sugar is present. Preparations in which less than 1/2 of –COOH groups are in methyl ester form (< 7 %) are called low methoxy pectins. Low methoxyl pectins gels only in presence of divalent cations and can form a gel even if percentage of solids are very low. Low methoxy pectins do not require the presence of sugar for the formation of gel. The divalent ions (Ca^{2+}) react with carboxyl group on the molecules of pectic acid and form a bridge between them.

c) pH: Most pectic products do not form jellies until pH is lowered to 3.5. The firmness of jellies increases as pH decreases. With very low pH, the amount of pectin required is very less and satisfactory gel still formed.

d) Sugar: Sugar is necessary for formation of pectin gels and must be present in minimum concentration. Most jellies are made with 65% of sugar. If amount is increased above 65%, crystallization tends to occur on jelly's surface and occasionally even within the jelly.

27.3. PICKLES

Pickles are easy to prepare with right ingredients and can be preserved for months. Pickles serve as a flavor enhancer and consumed typically in small quantities along with usual meal. They add to palatability of a meal, aid in digestion and are good appetizers. There are a wide variety of different pickles made and each is usually made with a mixture of fruits or vegetables which are chopped and immersed in a liquid (often oil or lemon juice) and a

variety of different spices and salt. Varieties of pickles include, pickles from lemon, mango, *amla*, ginger, green chilly, mix vegetables, cucumber, cabbage, garlic, carrot and sometimes fish, prawns, eggs or meat etc.

Addition of salt and acid to pickle gives the food a salty or sour taste. Most distinguishing characteristic of pickle is a low pH i.e. pH 4.6. This prevents the bacterial spoilage of foods and preserves perishable foods for months. Antimicrobial herbs and spices, such as mustard seed, garlic, cinnamon or cloves, are also added to pickles. Edible oils can also play a part as an oxygen-excluding covering for pre-pickled matter.

27.3.1. Types of pickling

a) Long, fermentation-based pickling: Requires a 'curing' period (up to several weeks) at room temperature.

b) Quick, unfermented pickling: It is made by adding acid (e.g. vinegar) to prevent bacterial growth.

27.3.2. Classification of pickles

a) Acid-based Pickles: The most common liquid for acid pickling is vinegar. This is an impure, dilute solution of acetic acid, obtained by the fermentation. Examples of vinegar-based pickles are pickled ginger, pickled vegetables (a mixture of onions, carrots, cauliflower, etc.), pickled sausages, etc.

b) Dry-salted Pickles: Salt has two effects when added to fruit or vegetables. It draws water from them by osmosis and triggers the fermentation process of the lactic bacteria. The resultant fermentation produces a particularly rich range of complex flavours. The most common dry-cured pickle is sauerkraut, dry salted pickled limes and lemons, plums, etc.

c) Brine-based Pickles: Brine-pickling works by a combination of osmosis and lactic fermentation. Cucumbers are traditionally pickled in brine along with other flavourings. Other examples are brine-pickled vegetables, garlic, chillies, etc.

d) Lye Pickling: Olives cannot be eaten in their raw state and require pickling to render them digestible. Before pickling, they require treatment with lye to remove substances which would be toxic to the bacteria causing fermentation. Once pickled, olives are packaged in various forms, with the addition of various herbs and spices, in brine, vinegar, and oil or dried and salted.

e) Pickles in Sugar: Fruits are sometimes first pickled (using vinegar) before being stored in a syrup or honey. Alternately, sweet-sour syrup is often made by adding sugar to vinegar. Such pickles are normally served along with meats or cheeses. Examples are watermelon rinds, walnuts, etc.

f) Oily Pickles: Oil finds its way into pickles. Various species of mushroom which are brine-pickled before storage in olive oil. Mustard oil and other vegetable oils are added to dry salted fruit and vegetables (lemon, mangoes, chillies, etc) along with spices.

27.3.2. Preservation principles

Commercial preservation of many pickles relied upon conversion of fermentable carbohydrates to organic acids during bulk storage and/or the addition of sufficient amount of sugar, vinegar and other ingredients to the fully cured and packed products to preclude any microbial growth. Organic acids, oils, salt and spices all have antimicrobial properties at suitable concentration especially in combinations which preserve the pickles. Pickles

in brine as such or after fermentation however, need some amount of preservative or pasteurization to prevent the spoilage.

27.3.3 Pickling process

Pickling is done in two stages:

1. By curing or fermentation with dry salting or fermentation in brine or salting without fermentation.
2. By finishing and packing.

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Lesson 28

Classification, composition and gradation

28.1. INTRODUCTION

A beverage is any product that is used as a drink, for the purpose of relieving thirst and introducing fluids in the body, nourishing the body and stimulating or soothing the individual.

28.2. CLASSIFICATION

The beverages can be classified as follow:

1. **Carbonated beverages**

   Alcoholic: Contains alcohol. e.g. beer

   Non-alcoholic: Contains no alcohol. e.g. soft drinks

2. **Non-carbonated beverages**

   Alcoholic: Contains alcohol. e.g. wine

   Non-alcoholic: Contains no alcohol. e.g. tea, coffee, cocoa

Alcoholic Beverages:

Alcoholic beverages are, in essence, flavored solutions of ethanol. The flavors may come from grains (e.g. Beer) or from grapes and other fruit (e.g. Wine) or from any source of carbohydrates, grains, sugar, or grapes (e.g. whiskey, rum, and brandy).

Non- alcoholic Beverages:

These types of beverages include the fruit juices, tea, coffee, cocoa/chocolate drinks etc. In order to enhance their thirst quenching and refreshing properties, some drinks are carbonated. Carbonated fruit based drinks are new age beverages which provide nutritional elements of the fruit along with natural colour and flavour in addition to carbonation effects.

Beverages may also be classified according to their function in the body. A particular beverage may have more than one function.

1. Refreshing

    i) Plain water

    ii) Carbonated beverage containing no fruit juice

    iii) Buttermilk with spices

2. Nourishing

    i) Milk and milk shakes

    ii) Fruit juices

    iii) Glucose, lemonade-it refers to noncarbonated soft drink made of a mixture of lemon juice, sugar and water.

3. Stimulating

    i) Egg nogs

    ii) Coffee or tea

    iii) Cocoa or chocolate beverages

4. Soothing

    i) Warm milk

5. Appetizing

    i) Soups

    ii) Fruit juices

28.3. COMPOSITION AND GRADATION OF TEA

28.3.1. Composition of tea A representative analysis of fresh leaf flush is presented in Table given below. Compositional data referring to fresh leaf are based on dry-leaf solids, since leaf moisture varies from 75 to 80%.

Table- 28.1: COMPOSITION OF GREEN TEA

Constituents	% (on dry weight basis)
Flavanols	25.0
Flavonols and flavonol glycosides	3.0
Polyphenolic acids and depsides	5.0
Other polyphenols	3.0
Caffeine	3.0
Theobromine	0.2
Amino Acids	4.0
Organic acids	0.5
Monosaccharide	4.0
Polysaccharides	13.0
Cellulose	7.0
Protein	15.0
Lignin	6.0
Lipids	3.0
Chlorophyll and other pigments	0.5
Ash (mineral matter)	5.0
Volatiles components	0.1

28.3.2. Grades of tea

The numerous grades of tea found in the trade are defined by their origin, climate, age, processing method and leaf grade. They can be classified as follows:

1. According to the leaf grade: tea with intact leaves

⬜⬜**Flowery Orange Pekoe and Orange Pekoe**: leaf buds and the two youngest leaves.

⬜⬜**Pekoe**: It includes leaf buds and the three youngest leaves.

⬜⬜**Pekoe souchong**: includes up to coarsest sixth leaf in the young twig.

2. Broken tea: are those with broken or cut leaves similar to above grades in which the fine broken or cut teas with the outermost golden leaf tips are distinguished from the coarse, broken leaves. Broken tea is the preferred product in world trade.

3. Fannings: Small fragments of the broken leaves. It is freed from stalks and stem. Used preferentially for manufacture of tea bags.

4. Tea dust:

5. Brick tea: The tea dust is compressed into a molded brick by sifting, steaming and pressing in the presence of a binder into a stiff, compact tea bricks and portions of it are broken off for use.

28.4. COMPOSITION OF ROASTED COFFEE

The composition of roasted coffee varies depending on the variety and extent of roasting. Several new flavouring constituents are generated during this process.

Table 28.2. The composition of *coffea arabica* after normal roasting

<u>Constituents</u>	Content (%)
Lipids	13.0
Protein	9.0
Polysaccharide, water insoluble	24.6
Polysaccharide, water soluble	6.0
Saccharose	0.2
Glucose, Fructose, Arabinose	0.1

Chlorogenic Acids	3.7
Caffeine	1.2
Trigonelline	0.4
Nicotinic Acid	0.92
Volatile Aroma Compounds	0.1
Formic Acid	0.1
Acetic Acid	0.25
Non-Volatile Acids(lactic, pyruvic, oxalic, tartaric and citric)	0.4
Minerals (ash)	4.0
Unidentified constituents	35.0
Moisture	2.5

28.4.1. Grading of coffee beans

Quality of Green Coffee is based on the odor and taste assays as well as on the size, shape, colours, hardness and cross-section of the beans. The plantation grades of cured coffee are:

1. Pea Berry (oval shaped beans)
2. O or A (first size in flats – bold, heavy and well formed)
3. B (slightly smaller than O or A)
4. C (slightly smaller than B)
5. Triage (pale, discolored beans)

A. COMPOSITION OF CACAO BEANS

The compositions of fermented and air-dried cacao nib, cacao shell and germ are presented in Table

Table- 28.3: Composition of the fermented and air-dried cacao nib or beans

Constituents	Content (%)
Lipids	54
Caffeine	0.2
Theobromine	1.2
Polyhydroxyphenols	6.0
Crude protein	11.5
Mono & oligosaccharides	1.0
Starch	6.0
Pentosans	1.5
Cellulose	9.0
Carboxylic acids	1.5
Ash	2.6
Moisture	5
Other components	0.5

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Lesson 29.A

Chemical changes during processing – Tea

29. B. 1. INTRODUCTION

Tea is second only to water in worldwide consumption. The scientific interest in tea is due in part to the unusual chemical composition of its leaf and the complex series of reactions that occur when these components are converted to those found in commercially available dry tea. "Tea and tea brew" refers only to the plant *Camellia sinensis*. Tea/tea blends are considered to be the tender shoots of tea shrubs consisting of young leaves and the bud. Tea is processed in a way traditional to country of origin.

Two most widely cultivated & important varieties of the species of tea are:

1. *Camellia sinensis var. sinensis* - with small leaf size (Chinese variety)

2. *Camellia sinensis var. assamica* - with bigger leaf size

29. B. 2. TYPES OF TEA

There are three types of tea depending on the processing of the tea leaves.

1. **Black tea** – Fermented type of tea in which flavanol oxidation is desirable.
2. **Green tea** –a dry product exhibiting the desirable twisted leaf appearance, but without flavanol oxidation. Because of the presence of unoxidized catechins, green tea beverage is yellow-green in color and more astringent than black tea.
3. **Oolong tea** - only partially oxidized, so that its appearance and chemical composition is somewhat intermediate between that of green and black tea.

29. B. 3. BLACK TEA MANUFACTURING

The processing of tea flush to black tea comprises the following major steps:

1. Withering

2. Preconditioning

3. Rolling

4. Fermentation

5. Firing

29.B.3.1. WITHERING

All the steps in black tea manufacturing are designed to accelerate the oxidation of the tea flavanols and effectively control the reactions to produce the end products with optimized flavor and leaf & beverage appearance. Fresh leaf is brought to the factory after harvesting. It is then subjected to a withering step to reduce leaf moisture from 75-80% to 55-65%. Withered leaf is flaccid and can be worked further without excessive fracture. Leaf is spread in 8 to 10 cm layers on nylon netting occupying a high proportion of total factory space. Warm air from the tea-drying ovens is usually circulated across the beds to facilitate evaporation. Depending on the system used and the prevailing weather conditions, the withering process takes 6 to 18 h during which time, moisture content of leaf drops either to 60% called soft withering or up to 50% called hard withering. In this stage, the leaf acquires a "kid glove feel" (i.e. that of a goat baby). Significant chemical changes begin at this step. Cell membranes become more permeable and level of caffeine, amino acid, and organic acid increases. The end point of withering is usually determined by experienced observation of leaf texture or sometimes by checking the weight loss of an isolated portion of leaf.

29.B.3.2. PRECONDITIONING

Preconditioning of the withered tea leaf consists of rolling the leaf 10-15 min. without application of any pressure. The purpose of tea conditioning is to impart the desired twist and compactness to the leaf and also to make available maximum amount of the polyphenol oxidase. Care is to be taken that destructing of tea leaves do not take place.

29.B.3.3. ROLLING

The "rolling" or leaf maceration step is carried out in order to disrupt cell structure and allow contact between tea flavanols and tea polyphenol oxidase. The physical condition of the leaf mass must also facilitate oxygen availability. Orthodox rolling takes place on a rotating circular table 1 to 1.3 m in diameter that is equipped with battens. Rolled leaf has a coating of leaf juices on the surface and a moist, fluffy texture. It is desirable to prevent leaf temperature from rising above 35 ℃ during the maceration process to preserve quality. Additionally new reactions that are the important for black tea characteristics are themselves initiated by more intimate mixing of

the leaf constituents. These reactions continue during rolling and are allowed to proceed to the desired extent in the fermentation stage of tea processing.

29.B.3.4. FERMENTATION

The oxidative process actually starts with the onset of maceration of withered leaf. At the end of the rolling process leaf is allowed to oxidize in 5 to 8 cm beds on trays in another fermentation room. It is desirable to keep temperatures below 30ºC. Oxidation at 15 to 20°C is said to improve flavor. Oxidation time depends on the temperature, degree of maceration, degree of wither, and the type of tea to be produced. It ranges from 45 min to 3 h. During this period tea leaves change colour from green to coppery red and aroma changes from grassy to sweet and flowery – an indication of end of fermentation. A slight loss of extractable caffeine at the level of 5-7 % is observed. Some of the reactions occurring during fermentation are enzyme catalyzed and of these the most important are:

1. Oxidation of tea flavanols by polyphenol oxidase - leads to development of colour, strength and quality of tea, brews.

2. Occurrence of reactions – leads to characteristic aroma of black tea

Chemistry of Tea Oxidation

Tea oxidation is generally referred to as "fermentation" because of the erroneous early conception of black tea production as a microbial process. Not until 1901 was there recognition of the process as one dependent on an enzyme catalyzed oxidation. This step and further reactions result in the conversion of the colorless flavanols to a complex mixture of orange-yellow to red-brown substances and an increase in the amount and variety of volatile compounds. Extract of oxidized leaf is amber-colored and less astringent than the light yellow-green extract of fresh leaf and the flavor profile is considerably more complex.

Flavanol Oxidation

The initial oxidation of the flavanol components of fresh leaf to quinone structures through the mediation of tea polyphenol oxidase is the essential driving force in the production of black tea.

Theaflavin formation

The oxygen-consuming reaction between a quinone derived from a simple catechin and a quinone derived from a gallocatechin results in the formation of a theaflavin. Theaflavins are orange-red substances that contribute significantly to the desirable appearance of black tea beverage. Although theaflavin content is considered to be an

important criterion of black tea quality, it does not exceed 2% of final product (dry leaf) weight and therefore only accounts for 10%, at the most, of the original catechin content of the leaf. Theaflavin content increases initially as the oxidation process proceeds but falls off rapidly on prolonged oxidation.

Structures of major tea catechins
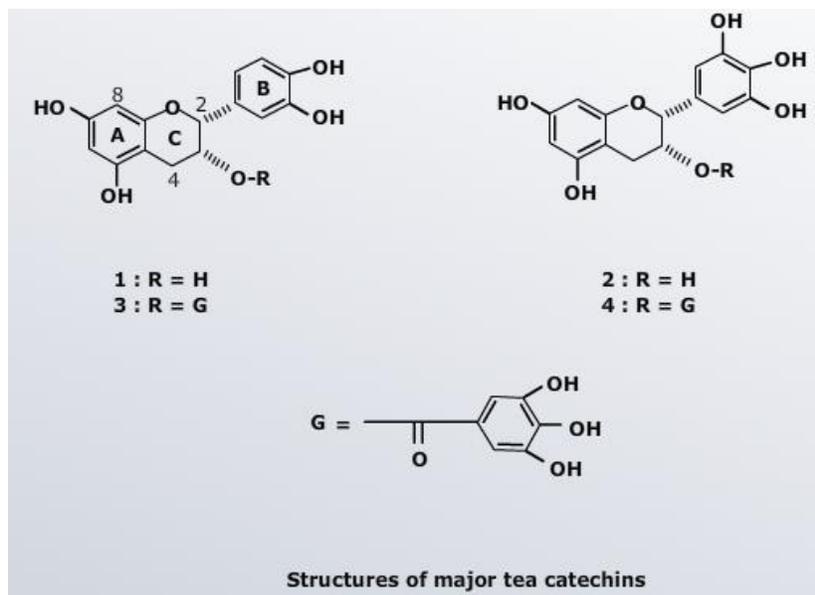
Fig-29.B.1: Structure of major catechins

Bisflavanol formation

Bisflavanols are the compounds formed by the coupling of the quinines produced by the oxidation of epigallocatechin and epigallocatechin gallate. The three predicted bisflavanols have been found and characterized in black tea. They occur only in very small quantities in black tea, presumably because of high reactivity.
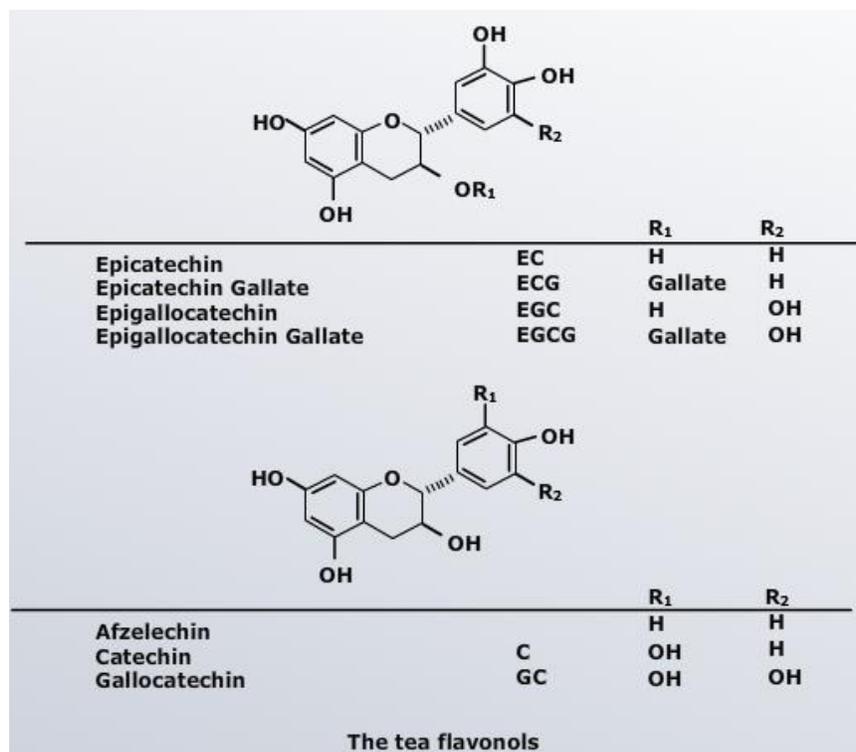
Fig- 29.B.2: The tea flavanols

Thearubigen formation

Approximately 15% of the original fresh-leaf flavanol fraction is recoverable from black tea, and about 10% is identifiable in the form of theaflavins and bisflavanols. While some flavanol material may become tightly bound to the insoluble portion of the leaf, the major amount is found in a complex mixture of only partially resolved substances known as thearubigens because of their red-brown color. Theaflavin levels decrease during prolonged oxidation of tea leaf. In addition, it is possible that chlorogenic acid, theogallin, and the flavonol glycosides are also oxidized by the quinones and become included in the thearubigen fraction. Thearubigen should be considered a sensory parameter, useful in qualitative assesment of the progress of tea fermentation.

Fig-29.B.3: Catechins oxidation

Epicatechins, Epigallocatechins & their gallates

O_2 | poly phenol oxidase

Oxidized epicatechin ← (tannins)
(O-quinones of epicatechins)

Theaflavin   bis-flavanol   thearubigins   epitheflevic acid
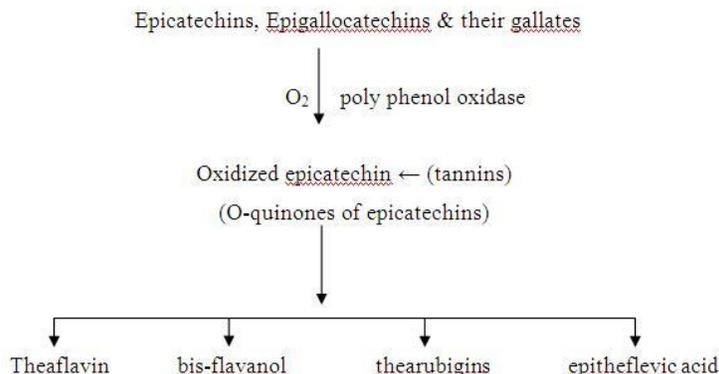
The enzymatic oxidation of flavanols via corresponding to o-quinones gives theaflavin (bright red colour and good solubility), bis-flavanols (colourless) and epitheaflavin acid (bright red colour & excellent solubility). Thus the theaflavins and epitheaflavins acid are the important derivatives that impart colour to the black tea. Apart from these, the theaflavins also impart the properties of quality & brightness of colour to tea brew. Another heterogeneous groups of compounds found in tea after enzymatic oxidation of flavanols is the thearubigins. This group of compounds is responsible for the characteristic reddish yellow colour and astringent taste of black tea extract. Thus, the thearubigins make an important contribution to colour, strength and mouthfeel of tea liquors.

29.B.3.5. FIRING

It is the final stage of tea processing, when the rolled and fermented leaf having moisture of 45-50% is dried to produce black tea containing 3% moisture. It is usually accomplished by passing trays of fermented leaf through a hot-air dryer (inlet temperature of 87-90 °C and outlet temperature of 56-57 °C) in a countercurrent mode. The drying process takes about 20 min. Control over time –temperature during firing is crucial to final product quality. A noticeable effect of firing is the change of color brought by the transformation of chlorophyll to pheophytin, which imparts the desired black color to the dried product. Reduction of astringency of fermented leaf due to reaction of poly phenol with tea leaves proteins at elevated temperature. This leads to mellowing of flavour during firing. Much of the characteristic black tea aroma is generated during firing. Some low-boiling fresh leaf volatiles are lost but many new components are generated. Firing results in the loss of small amounts of caffeine through sublimation.

Lesson 29.B

Chemical changes during processing – coffee

29.B.1. INTRODUCTION

Coffee or coffee beans are the seeds of fruits from which various layers surrounding the beans are successively removed during its processing. The seeds are used as raw/roasted, whole/ground and should be obtained from the botanical genus *Coffea*. The beverage prepared from such seeds is called as coffee. Coffee drink is a variable and complex beverage.

The two main species of commercial interest in the genus *Coffea* are *Coffea arabica* and *Coffea canephora* var. *robusta.* They are also known as Arabica and Robusta, respectively.

Coffee is grown in countries situated between the Tropics of Capricorn and Cancer. Its native is African continent – mainly Ethiopia. Brazil is the largest coffee producing country in the world. Arabia, Java and Venezuela are also producing coffee with special grade of their choice.

The widely cultivated species of the coffee are:

1. *Coffea arabica* - provides 75% of the total world's coffee production.

   Major coffee producing states in India are Tamil Nadu, Karnataka, Kerala and Orissa.

2. *Coffea canephora* - provides about 25% of the world's coffee production.
3. *Coffea liberica* –provides less than1% of the world's coffee production.

All varieties of *Coffea canephora* are marketed under the common name "robusta*".*

The coffee berry in a cross-sectional diagram.
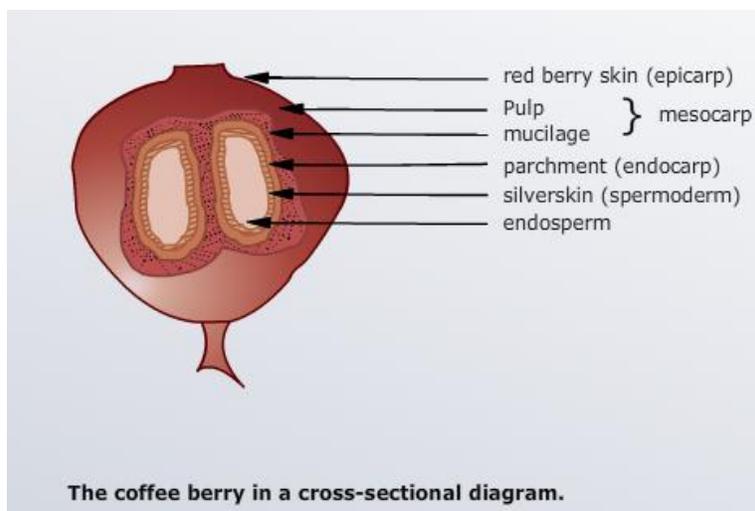
Fig-29.A.1: The coffee berry in a cross- sectional diagram

29.A.2. METHODS OF PROCESSING OF GREEN COFFEE

The processing of coffee involves removal of various layers. The outermost red skin, also known as epicarp, is completely removed. The pulp of coffee berry is then removed which is followed by removal of mucilage. These two layers are collectively known as mesocarp. Then Parchment layer (endocarp) is removed. Finally, the silver skin (endosperm) surrounding the green coffee berry is removed. Two methods are employed for coffee processing.

1. Dry method - natural method

2. Wet method - washing method

1. Dry method - natural method

This method is widely used in Brazil. It involves sun drying until the beans get separated by shrinking from the surrounding parchment layer. This is followed by dehulling and polishing of the beans. Sometimes, the fresh beans are piled up, allowed to ferment the fruity pulp of beans for 3-4 days under their own heat. Then the beans are dried to remove the husks and parchment from the dried berries, and as much as possible of silver skin. The dried and dehulled beans are then polished. The dehulled and cleaned coffee beans are then classified according to size/shape and packed in bags.

2. Wet method - washing method

In the wet process, the uniformly ripe and freshly harvested coffee berries are brought to a pulper, wherein the fruits are rubbed between a rotating cylinder and a slotted plate. The passage of fruit produces a rubbing action, which facilitates the removal of the skin and pulp from the beans without any damage to the seeds. The beans obtained from pulper have the silver skin, the parchment layer and a mucilage layer. Coffee beans are then carried into water fermentation tanks and beans are left to ferment for 12-48 hours. During fermentation, the mucilaginous layer is hydrolyzed by enzymes of the coffee and other enzymes produced by the microorganisms. The mucilage is degraded to large extent and can be readily removed by washing with water. The beans may be then sun-dried or with the help of mechanical dryers (hot air 65-85 °C). Beans are still covered by parchment shell which is removed by dehulling process. Finally, beans are polished to a smooth, glossy surface.

The wet (washing) method of coffee processing is more sophisticated than dry method, and produce better quality coffee. Almost half of the world coffee is processed by the wet method.

29.A.3. ROASTING OF GREEN COFFEE BEANS

Green berries, after the removal of various layers, smell green-earthy. To bring about the delightful aroma of coffee, they must be heat treated in a process called roasting. The roasting process is characterized by a decrease in old compound and generating new compounds. Green coffee contains normally 12% moisture. Most of the moisture is lost during early stages of roasting process, followed by a loss of up to 8% of the dry beans. The style of roasting process is a matter of personal taste and liking of flavour of finally roasted beans. Some Arabica coffees become bitter if high a temperature of roasting is employed. Hot gases at about 200-250°C are used to roast the beans in a perforated steel rotating drum. Beans are heated in small quantities for a total of only 5 min. When the beans have reached to the desirable internal temperature and colour, they are moved away from the oven. The roasted beans are immediately cooled, with fine water spray, to prevent the further pyrolytic changes in the bean.

In general, the changes taking place during roasting of coffee beans are:

ü The beans increase in volume (50-80%)

ü The colour changes from green to brown

ü 13-20% loss in weight of beans

ü Build-up of the typical roasted flavour of the beans

ü Specific gravity of beans falls drastically from 1.126-1.272 to 0.570-0.694

ü The structure of the beans changes. The horny, tough and difficult to crack beans become brittle and mellow after roasting.

29.A.4. ROASTING PROCESS IS DISTINGUISHED INTO FOUR MAJOR PHASES:

1. Drying
2. Development
3. Decomposition
4. Full roasting

The initial changes during roasting process occur at or above 50°C when the protein present in the tissue cells gets denatured and evaporation of water takes place. At above 100°C temperature, browning reactions take place due to pyrolysis of organic compounds. Volatile products (water, CO, CO_2) are release at about 150°C resulting in an increase in bean volume. Part of the CO_2 produced gets absorbed within the texture of the roasted beans.

During decomposition phase, at 150-200°C, beans are being forced to pop and burst with formation of bluish smoke and subsequent release of coffee aroma. During roasting, pressure develops in the beans which hold the initial breakdown products together. When the proper stage of roasting is reached, these breakdown products react with each other to produce coffee flavour. Finally, the full roasting phase is achieved, in which the moisture level of the beans drops to the final level of 1.5 - 3.5%.

29.A.5. CHANGES IN INDIVIDUAL CONSTITUENTS DURING COFFEE PROCESSING

1. **Proteins**: Extensive changes taking place in protein of coffee beans during roasting. This happens in the presence of carbohydrates. A shift in the amino acid composition of coffee protein acid hydrolysates is observed before and after bean roasting. A drop of about 30% in the total amino acid content of the hydrolysate take place because of considerable degradation during roasting.

2. **Carbohydrates**: During roasting, a high amount of the carbohydrates, most of the sucrose and monosaccharides are degraded and decomposed. The carbohydrates are also involved in the caramelization process.

3. **Lipids**: A little change observed in the lipid fraction. It remains stable and survives the roasting process with some minor changes. The diterpene glycosides (Cafestol and kahweol) are degraded during roasting and their residues get entry into the soluble coffee powder and subsequently in coffee beverage to contribute to the bitter taste of coffee beverage.

4. **Acids:** Chlorogenic acids are the most abundant acids of coffee. Formic and acetic acids predominate among the volatile acids. The non-volatile acids are lactic, tartaric, pyruvic and citric acids. During roasting process, these acids are decomposed by about 30 -70%.

5. **Caffeine**: The caffeine level in beans is only slightly decreased during roasting. It is mildly bitter in taste with a threshold value in water is 0.8–1.2 mmole∕l. It is the best known N-compound having physiological effects like stimulation of the central nervous system, increased blood circulation and respiration, etc.

6. **Trigonelline and nicotinic acid:** It is present in green coffee upto 0.6%. About half quantity of these compounds are decomposed during roasting. The degradation products include nicotinic acid, pyridine, their methyl esters, etc.

7. **Aroma Compounds**: The aroma of coffee is not stable. The aroma profile of coffee is of sweet/caramel-like, earthy, sulfurous/roasty and smoky/phenolic. The volatile compounds of roasted coffee have very complex composition.

   Ø The group of aliphatic compounds includes hydrocarbons, alcohols, and above all, carbonyl compounds, which are derived during roasting from carbohydrate fragmentation. Also numerous alicyclic compounds are found.

   Ø Phenols are predominant among the aromatic compounds and are most probably derived from thermal decomposition of chlorogenic acids; Phenol ethers, carbonyl esters and polycyclic compounds are also present.

   Ø There is a large number of heterocyclic compounds, among which are many 2- and 2,5- substituted furans, probably derived from the pyrolysis of sucrose and other sugars.

8. **Other Constituents**:

   Brown pigments (melanoidins) are derived from Maillard reactions or from carbohydrate caramelization. Apparently, cholrogenic acid is also involved in such browning reactions. Secondary products of the degradation of mixtures of carbohydrates and proteins are probably involved in the formation of the bitter flavour of roasted coffee.

29.A.6. GRINDING AND STORAGE OF ROASTED COFFEE

The grinding of roasted beans is generally done in industrial grinders like roll and breaker bar systems. The roasted beans are rolled past the toothed rollers, where they are cut. A series of these rollers produces a successively finer grind.

Other systems used on a large scale include hammer mills with cutting blades. The roasted beans should be cool, hard, and brittle. Dark roasted beans grind the most readily. Large amounts of carbon dioxide are released from roasted coffee, along with other volatile compounds, during this process. This provides temporary protection to the freshly ground coffee from the atmospheric oxygen and moisture in the air. Ground coffee is usually packed within 8 h of grinding. Storage in evacuated and sealed container at -20°C prevents from staling.

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Lesson 29.C

Chemical changes during processing – Cocoa

29.C.1. INTRODUCTION

Cocoa is indigenous to South America and is believed to have originated from the Amazon valley and Orinoco valley. Cocoa beans are the seeds of the tropical cacao tree *Theobroma cocoa* family *sterculiaceace*. Cocoa drink is different from tea or coffee as it is consumed as a suspension and not in the form of an aqueous extract. Cocoa and its products contain stimulating alkaloids, ex. theobromine; with substantial amounts of fats, carbohydrates and proteins. Unlike coffee and tea, cocoa drinks are to be consumed in large quantity to have a stimulating effect.

29.C.2. CLASSIFICATION

1. Criollo (means native): bears highly aromatic beans, hence their commercial name "flavor beans". It was probably cultivated first in the region from southern Mexico.

2. Forastero (means foreign): less flavorful beans. Most important commercial type of cacao and accounts for the bulk of world cacao production. It has its origins in the upper Amazon basin area of northern Brazil, eastern Venezuela and Colombia.

3. Trinitario ("of Trinidad") is a cross between Criollo and Forastero which probably occurred naturally.

4. Nacional (or Arriba) is scientifically a Forastero but is classified separately due to its distinctive aromatic floral nature.

29. C. 3. PROCESSING OF COCOA

On farm processing of cocoa consists of fermentation and drying. The bulk of the harvest is fermented before drying.

1. Fermentation of cocoa

The cocoa beans along with the pulp are fermented for 2-8 days. Cocoa fermentation is necessary for:

1. Formation for the flavour precursors
2. Killing the beans to prevent cocoa butter degradation.
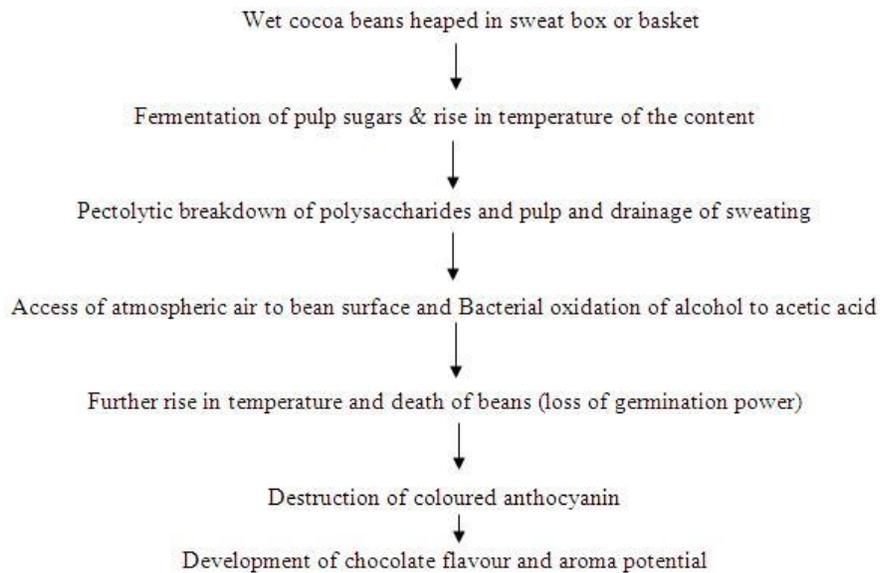3. Degradation of the fruit pulp to make bean drying easier.

Fig- 29.C.1: The major changes during fermentation of cocoa

The fermentation of cacao beans is accomplished by two processes

1. External microbial processes

2. Internal autolytic processes

The microbial processes are characterized by the production of the ethanol and acetic acid. The autolytic process involves changes due to cocoa beans enzymes. The group of microorganisms involve in the fermentation are mainly yeast, lactic acid bacteria, acetic acid bacteria and spore forming microorganisms.

1. External microbial process

ü The pulp sugar is fermented by yeast to alcohol and carbon dioxide on the first day.

ü Pectolytic enzymes and other glycosidases cause degradation of the polysaccharides. This results in liquefaction of the fruit pulp, which is drained away.

ü As a result aeration of the bean sim proves. This causes oxidation of alcohol to acetic acid by the acetic acid bacteria during second to fourth day. These processes cause the temperature of the cacao mass to rise to about 45-50^0C. At the same time the pH of the pulp rises from an initial value of 3.5-4.0 to around a pH of 5.0. Simultaneously, the pH of the cotyledons drops from about 6.5 to a value of 4.5.

ü The cell walls become permeable and the cacao seed is killed. The causing the death of beans has been identified as acetic acid. After the bean death, the oxidative processes take over the entire mass.

2. Internal autolytic process

It occurs during 5th to 7th day of fermentation. The enzymatic changes in cocoa beans can be divided into two phases: Anaerobic hydrolytic phase and Aerobic oxidative phase. The changes occur during processing are:

1. The glycosidase splits the anthocyanins to cause bleaching of the violet colors of the tissues under anaerobic conditions.

2. Certain proteases which are active during anaerobic hydrolytic stage of the fermentation cause changes in the proportion of the proteins and amino acids of the fermented cocoa beans.

3. Polyphenol oxidase present causes oxidation of the epicatechins to quinone. Thus quinone is capable of complexing with itself or other quinones to produce a brown substance. The oxidizedepicatechins also cause irreversible tanning of the proteins in cocoa beans.

4. Reaction of the polyphenols decreases the soluble phenol content and thereby, mellows the original harsh and astringent cocoa flavours.

29. C. 4. CHANGES TAKING PLACE INSPECIFIC CONSTITUENTS

1. Carbohydrates

Total sugar content decreases throughout the fermentation. During fermentation, sucrose hydrolysis provides the reducing sugar pool, which is important for aroma formation during the roasting process.

2. Proteins

Proteolysis begins soon after initiation of fermentation and continues throughout the fermentation period. A definite decrease in the protein nitrogen content along with an increase in the peptides and free amino acid occurs. Tanning of enzymes of cocoa beans has also been found during fermentation.

3. Lipids

The fat does not change appreciably during fermentation.

4. Acids

Organic acids in cocoa beans are formed mainly during cocoa fermentation and consist mainly of acetic acid (a flavour constituent), citric acid and oxalic acid. Of total acids (1.2-1.64%),0.79-1.25% is volatile acids and 0.19-0.7% is acetic acid. Acetic acid and to a lesser extent the volatile acids and non-volatile acids contribute to final chocolate flavour.

29. C. 5. CHANGES TAKING PLACE DURINGDRYING PROCESS

The browning of the cotyledons is considered to be most important change during drying. The polyphenolic brown pigments are formed as a result of polyphenol oxidation. By the end of drying, oxidation reaction is terminated and moisture content of the beans drops to about 8%.

29. C. 6. CHANGES DURING ROASTING PROCESS

Roasting is the processing step generally done by the consuming countries. Generally roasting temperature should not exceed 150^0C.The major changes taking place during roasting are:

1. Reduction in the moisture content to about 3%

2. Contribution to the further oxidation of phenolic compounds.

3. Causes removal of the acetic acid, volatile esters and undesirable aroma compounds.

4. Enhanced aroma of the beans: The typical aroma of the roasted nuts is derived from pyrazine; the bitter taste is derived from purines, theobromine and caffeine.

5. The seed hardens and becomes more brittle. The shell is loosened and easily removed.

6. The color of the beans deepens.

7. The reducing sugars and amino acids in cocoa beans react via mail lard's reaction and strecker's degradation reaction to form many of the components found in chocolate flavour.

8. The ammonia liberated during roasting can react with reducing sugar or other compounds to produce compounds important to the flavour of the chocolate.

9. The level of the monocarbonylcompounds changes.

10. The overall loses induced by roastingare 5-8%

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Lesson 30

Determination of total ash, alkalinity of water-soluble ash and water extractives in tea and detection of chicory in coffee powder.

30.1. Problem: To determine the water extractives in tea leaves

Instant tea processing begins with extraction of tea (mostly black tea). The extract represents approximately 85% of the soluble solids in the tea leaves. Tea extract contains both caffeine and tannins (polyphenols) which are soluble in hot water, but some tannins form haze in cold water. Some "instant tea" manufacturers add dextrin in the extract prior to drying. Approximately 30% of the dry weight of tea leaves is made up of polyphenols formerly known as "tea tannins". The sugars found in fresh tea flush are glucose, fructose, sucrose together with traces of raffinose and stachyose. Fresh tea flush contains organic acids namely, oxalic, malic, citric, succinic and isoditric (in decreasing order). Copper is the main important mineral and is found as a constituent of tea catechol oxidase (12 to 15 ppm).

Apparatus:

1. Conical flasks
2. Evaporating dish
3. Air condenser
4. Desiccator
5. Hot air oven
6. Water bath
7. Volumetric flasks (250 ml)
8. Analytical balance

Principle:

Ground tea sample is suspended in hot water and extraction is carried out by slow boiling using an air condenser for preventing loss of volatiles. This boiled tea suspension is filtered and an aliquot of it is dried in two stages:

1) Partial drying on boiling water bath

2) Complete drying in a hot air oven.

The dry solids obtained are expressed as percentage water extractives of tea.

Procedure:

1. Take 2 g of ground tea sample (dried in oven at 100 °C for 6 h, passing through sieve no. 30 – AOAC) in 500 ml conical flask.

2. Add 150 ml hot water and connect it with 75 cm long tube (as air condenser).

3. Reflux over a low flame for 1 h rotating occasionally (heat very slowly to prevent evaporation losses).

4. Cool and dilute to 250 ml volume. Mix thoroughly and filter it.

5. Transfer 50 ml of the aliquot to a weighed evaporating dish and evaporate to dryness on water bath.

6. Cool in desiccator and weigh accurately.

7. Repeat this process of heating for 30 min., cooling in a desiccator and weighing until the loss in mass between two successive weighings is less than 1 mg.

8. Record the lowest mass and determine the tea extractives.

Observations

1. Weight of tea sample taken   = W g

2. Final volume of tea extract   = V ml

3. Aliquot taken for drying   = X ml

4. Weight of empty dry evaporating dish   = W_1 g

5. Weight of dish + dry tea extract   = W_2 g

Calculations

$$\% \text{ Water extractives} = \frac{(W2 - W1) \times V \times 100}{X \times W}$$

Results: The water extractive content in the tea leaves is _____%

30.2. Problem: To determine the total ash and alkalinity of water-soluble ash in tea

The ash content in many tea ranges between 5 and 6 %. In fresh tea the proportion of ash may be as high as 10%. As per BIS standards, A tea must contain 4 to 8 % (by mass) total ash and minimum 40% of which must be (water soluble ash in spent tea, however, the ash drops) below 3% and the ash itself is high in calcium. In genuine tea about 50% of the ash is water soluble ash and is found in the range of 3 to 3.5% whereas in exhausted teas (spent) it drops to about 0.5 %. The major element in tea minerals is potassium which is half the total mineral content. Thus, the soluble ash obtained from spent tea will have lower alkalinity value.'

Apparatus:

1. Burette
2. Funnel
3. Conical flasks
4. Beakers
5. Silica dish/crucible
6. Desiccator
7. Muffle furnace
8. Analytical balance

Reagents:

1. HCl (0.1 N)
2. Methyl orange indicator (0.1%)

Principle:

Total ash is obtained by igniting tea sample in muffle furnace until it is free from carbon. The ash obtained is mixed with water and heated to boiling and filtered through ash less filter paper. The filtrate and washings are

combined together and the alkalinity of soluble ash is determined by titrating against 0.1 N HCl using methyl orange as an indicator. The alkalinity of soluble ash is expressed as number of ml of 1 N acid per 100 g sample.

Procedure

A. For total ash

1. Take 5 g of sample in a tarred silica dish/crucible.

2. Heat it at 100 °C in an oven until moisture is expelled.

3. Incinerate it at as low a temperature as possible.

4. Place the dish in furnace at 525 ± 20 °C and leave until white ash is obtained (30 min).

5. Transfer the dish in a desiccator for cooling and weigh the dish.

6. Express the results as % total ash.

B. For alkalinity of water soluble ash

1. Add 10 ml of water to the ash obtained as above and heat almost to boiling.

2. Filter through ash less filter paper and wash the residue with hot water

until the combined filtrate and washings measure about 60 ml.

3. Cool the filtrate and washings and titrate with 0.1 N HCl using methyl

orange indicator.

4. Express alkalinity of soluble ash as ml of 0.1 N acid required for 100 g of

sample.

Observations:

A. For total ash

    1. Weight of silica dish = W_1 g

    2. Weight of silica dish + sample = W_2 g

    3. Weight of silica dish + ash = W_3 g

B. For alkalinity of water soluble ash

    1. Normality of HCl = N

    2. Titre value = V ml

Calculations:

% Total ash = $\underline{W_2\text{-}W_3}$ x 100

  $W_2 - W_1$

Alkalinity = $\underline{V \times N}$ x 100

 $W_2 - W_1$

Results:

The ash content in the given tea sample is _____%

The alkalinity of its water soluble ash is_____ ml of 1 N HCl per 100 g sample.

30.2. Problem: To determine the presence of chicory in coffee powder

Chicory is blended in coffee mainly for reducing the cost. As per BIS standards the roasted coffee – chicory mixture shall contain caffeine not less than 0.6 % and the aqueous extract shall be 35 to 50 % (on dry matter

basis). Under PFA rules the instant coffee powder containing chicory are required to be labelled as "mixed with chicory" or "blended with chicory".

Principle: There are a few methods used for detecting the presence of chicory in coffee powder which includes:

1. Microscopic method

Under the microscope the chicory shows numerous thin walled parenchymatous cells, lactiferous vessels and sieve tubes with transverse plates. There are also present large vessels with huge well defined pits. The coffee grain has an altogether different characteristic structure, enabling therefore, an easy distinction between the two.

2. Chemical method

Roasted coffee contains 1.9 to 2.6 % reducing sugars as compared to 25 to 27% in chicory. Thus the cupric reducing power of the coffee extract could be used for concluding the presence of chicory. The strained coffee extract is treated with excess of lead acetate and the precipitates are allowed to settle. A colourless supernatant indicates the absence of chicory.

Procedure:

1. Boil 10 g of the coffee powder with 250 ml of water.

2. Strain and add an excess of basic lead acetate.

3. Allow the precipitates to settle.

4. Note the presence of colour in the supernatant.

5. A coloured supernatant indicates the presence of chicory.

Lesson 31

General principles of food preservation-Physical methods

31.1. Introduction

Foods are mainly composed of biochemical compounds which are derived from plants and animals. Carbohydrates, proteins and fats are the major constituents of food. In addition, minor constituents such as minerals, vitamins, enzymes, acids, antioxidants, pigments, flavours are present. Foods are subject to physical, chemical, and biological deterioration. The major factors affecting food spoilage are

1) Growth and activities of microorganisms (bacteria, yeasts, and molds)

2) Activities of food enzymes and other chemical reactions within food itself

3) Infestation by insects, rodents

4) Inappropriate temperatures for a given food

5) Either the gain or loss of moisture

6) Reaction with oxygen

7) Light

The vast majority of instances of food spoilage can be attributed to one of two major causes: (1) the attack by microorganisms such as bacteria and molds, or (2) oxidation that causes the destruction of essential biochemical compounds and/or the destruction of plant and animal cells. Chemical and/or biochemical reactions results in decomposition of food- due to microbial growth. There is a adverse effect on appearance, flavour, texture, colour, consistence and/or nutritional quality of food.

31.2. Food preservation

Food preservation is the process of treating and handling food to stop or greatly slow down spoilage (loss of quality, edibility or nutritive value) caused or accelerated by micro-organisms. Preservation usually involves preventing the growth of bacteria, fungi, and other micro-organisms, as well as retarding the oxidation of fats which cause rancidity. It also includes processes to inhibit natural ageing and dis-colouration that can occur during food preparation such as the enzymatic browning reaction in apples after they are cut. Preservative for food may be defined as any chemical compound and/or process, when applied to food, retard alterations caused by the

growth of microorganisms or enable the physical properties, chemical composition and nutritive value to remain unaffected by microbial growth.

31.3. Principles of Food Preservation

The principles of various methods for food preservation are as

1) Prevention or delay of microbial decomposition

 - By keeping out microorganisms (asepsis)

 - By removal of microorganisms

 - By hindering the growth and activity of microorganisms (e.g. by low temperatures,

 drying, anaerobic conditions, or chemicals)

   - By killing the microorganisms (e.g. by heat or radiation)

2) Prevention or delay of self decomposition of the food

 - By destruction or inactivation of food enzymes (by blanching)

 - By prevention or delay of chemical reactions (By using antioxidant)

31.4. Methods of Food Preservation

Preservation of food is achieved by application of physical, chemical and/or biological methods are as follows:

Physical methods

- Cooling to

  $\rightarrow$ Low temperature refrigeration (0 to 7ºC) - preserves for shorter period (days) $\rightarrow$ Freezing - preserves for several months

- Heating $\rightarrow$ pasteurization, cooking, sterilization etc

- Exposure to ionizing radiation $\rightarrow$ U.V., γ, etc

- Application of high pressure

- Drying $\rightarrow$ removal of water to a level which does not support the growth of microorganism

Chemical methods

- Quite often it is either impossible or undesirable to employ conventional physical methods of the preservation.

- In such situation one has to opt for chemical methods of preservation.

- It involves application of chemical additives which act as antimicrobial agents.

Biological methods

Souring (fermentation) lactic and acetic acid, e.g. cheese and cultured milk.

31.4.1. Thermal Treatment

The term "thermal" refers to processes involving heat. Heating food is an effective way of preserving it because the great majority of harmful pathogens are killed at temperatures close to the boiling point of water. In this respect, heating foods is a form of food preservation comparable to that of freezing but much superior to it in its effectiveness. A preliminary step in many other forms of food preservation, especially forms that make use of packaging, is to heat the foods to temperatures sufficiently high to destroy pathogens.

In many cases, foods are actually cooked prior to their being packaged and stored. In other cases, cooking is neither appropriate nor necessary. The most familiar example of the latter situation is pasteurization. Conventional methods of pasteurization called for the heating of milk to a temperature between 145 and 149 °F (63 and 65 °C) for a period of about 30 minutes, and then cooling it to room temperature. In a more recent revision of that process, milk can also be "flash-pasteurized" by raising its temperature to about 160 °F (71 °C) for a minimum of 15 seconds, with equally successful results. A process known as ultrahighpasteurization uses even higher temperatures of the order of 194 to 266 °F (90 to 130 °C) for periods of a second or more.

31.4.2. Low Temperature

The lower the temperature, the slower will be chemical reactions, enzyme action, and microbial growth. Each microorganism present has an optimal temperature for growth and a minimal temperature below which it cannot multiply. As the temperature drops from this optimal temperature toward the minimal, the rate of growth of the organism decreases and is slowest at the minimal temperature. Cooler temperatures will prevent growth, but slow metabolic activity may continue. Most bacteria, yeasts, and molds grow best in the temperature range 16-38°C (except psychrotrophs). At temperatures below 10°C, growth is slow and becomes slower the colder it gets. The slowing of microbial activity with decreased temperatures is the principal behind refrigeration and freezing preservation.

31.4.3. Drying

One of the oldest methods of food preservation is by drying, which reduces water activity sufficiently to prevent or delay microbial growth. The term water activity is related to relative humidity. Relative humidity refers to the atmosphere surrounding a material or solution. Water activity is the ratio of vapour pressure of the solution to the vapour pressure of pure water at the same temperature. Under equilibrium conditions water activity equals RH/100. At the usual temperatures permitting microbial growth, most bacteria require a water activity as low as 0.90-1.00. Some yeasts and molds grow slowly at a water activity as low as 0.65. Food is dried either partially or

completely to preserve it against microbial spoilage.

31.4.4. Irradiation

The lethal effects of radiation on pathogens has been known for many years. The radiation used for food preservation is normally gamma radiation from radioactive isotopes or machine-generated x rays or electron beams. One of the first applications of radiation for food preservation was in the treatment of various kinds of herbs and spices, an application approved by the United States Food and Drug Administration

(FDA) in 1983. In 1985, the FDA extended its approval to the use of radiation for the treatment of pork as a means of destroying the pathogens that cause trichinosis. Experts predict that the ease and efficiency of food preservation by means of radiation will develop considerably in the future.

31.5. Preservation of food through irradiation

Radiation processing of food involves exposure of food to short wave radiation energy to achieve a specific purpose such as extension of shelf-life, insect disinfestation and elimination of food borne pathogens and parasites. In comparison with heat or chemical treatment, irradiation is considered a more effective and appropriate technology to destroy food borne pathogens. It offers a number of advantages to producers, processors, retailers and consumers. Radiation processing of food involves exposure of food to short wave radiation energy to achieve a specific purpose such as extension of shelf-life, insect disinfestation and elimination of food borne pathogens and parasites.

31.5.1. Type of Radiation

The type of radiation used in processing materials is limited to radiations from high energy gamma rays, X-rays and accelerated electrons. These radiations are also referred to as ionizing radiations because their energy is high enough to dislodge electrons from atoms and molecules and to convert them to electrically-charged particles called ions.

Gamma rays and X-rays, like radiowaves, microwaves, ultraviolet and visible light rays, form part of the electromagnetic spectrum and occur in the short-wavelength, high-energy region of the spectrum and have the greatest penetrating power. They have the same properties and

effects on materials, their origin being the main difference between them. X-rays with varying energies are generated by machines. Gamma rays with specific energies come from

the spontaneous disintegration of radionuclides.

Naturally occurring and man-made radionuclides, also called radioactive isotopes or radioisotopes, emit radiation as they spontaneously revert to a stable state. The time taken by a radionuclide to decay to half the level of

radioactivity originally present is known as its half-life, and is specific for each radionuclide of a particular element. Only certain radiation sources can be used in food irradiation. These are the radionuclides cobalt-60 or cesium-137; X-ray machines having a maximum energy of five million electron volts (MeV) (an electron volt is the amount of energy gained by an electron when it is accelerated by a potential of one volt in a vacuum); or electron accelerators having a maximum energy of 10 MeV. Energies from these radiation sources are too low to induce radioactivity in any material, including food.

31.5.2. Unit of Radiation Dose

Radiation dose is the quantity of radiation energy absorbed by the food as it passes through the radiation field during processing. It is measured using a unit called the Gray (Gy).

In early work the unit was the rad (1 Gy = 100 rads; 1 kGy =1000 Gy).

31.5.3. Application of Radiation processing of food

Interest in the practical application of the process is emerging for many reasons. High food losses caused by insect infestation, microbial contamination and spoilage; mounting concern over food borne diseases, harmful residues of chemical fumigants and the impact of these chemicals on the environment, the stiff standards of quality and quarantine restrictions in international trade are some of the reasons. Though irradiation alone can not solve all the problems of food preservation, it can play an important role in reducing post-harvest losses and use of chemical fumigants.

On the basis of radiation dose, applications of radiation can be classified into:

Low Dose Applications

Sprout inhibition in bulbs and tubers	0.03 - 0.15 kGy
Delay of fruit ripening	0.25 - 0.75 kGy
Insect disinfestation including quarantine treatment and elimination of food borne parasites	0.25 - 1 kGy

Medium Dose Applications

Reduction of spoilage microbes to improve shelf-life of meat, poultry and seafoods under refrigeration	1.5 - 3 kGy
Elimination of pathogenic microbes in fresh and frozen meat, poultry and seafoods	3 - 7 kGy
Reducing number of microorganisms in spices to improve hygienic quality	10 kGy

High Dose Applications

Sterilization of packaged meat, poultry and their products which are shelf-stable without refrigeration	25 - 70 kGy
Sterilization of hospital diets	25 - 70 kGy

(Source: http://www.barc.ernet.in)

31.5.4 Advantages and disadvantages of radiation processing of food

Advantages	Disadvantages
Radiation processing is a cold process and therefore, unlike heat, it can be used on agricultural commodities without changing their fresh-like character	Radiation processing is a need based technology and cannot be applied to all kinds of foods
Radiation processing dose not alter significantly nutritional value, flavour, texture and appearance of food	Radiation processing cannot make a bad or spoiled food look good.
Radiation using Cobalt-60 cannot induce any radioactivity in food and does not leave any harmful or toxic radioactive residues on foods as is the case with chemical fumigantsd	It cannot destroy already present pesticides and toxins in foods
Due to the highly penetrating nature of the radiation energy, it is a very effective method	Amenability of a particular food commodity to radiation processing has to be tested in a laboratory
Prepackaged foods can be treated for hygienization and improving shelf-life	Only those foods for which specific benefits are achieved by applying appropriate doses, and those duly permitted under the Prevention of Food Adulteration Act (PFA) Rules, 1955, can be processed by radiation.

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Lesson 32

Chemical preservation of food

32.1. Introduction

Preservative for food may be defined as any chemical compound and/or process, when applied to food, retard alterations caused by the growth of microorganisms or enable the physical properties, chemical composition and nutritive value to remain unaffected by microbial growth. Some chemicals have been used traditionally since several decades as direct or indirect inhibitors of microbial growth and are still widely used despite their limitations

The majority of food preservation operations used today also employ some kind of chemical additive to reduce spoilage. Of the many dozens of chemical additives available, all are designed either to kill or retard the growth of pathogens or to prevent or retard chemical reactions that result in the oxidation of foods.

Some familiar examples of the former class of food additives are sodium benzoate and benzoic acid; calcium, sodium propionate, and propionic acid; calcium, potassium, sodium sorbate, and sorbic acid; and sodium and potassium sulfite. Examples of the latter class of additives include calcium, sodium ascorbate, and ascorbic acid (vitamin C); butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT); lecithin; and sodium and potassium sulfite and sulphur dioxide.

32.2. Classification of preservatives

According to FSSA rules → class I and class II preservatives

Class I preservatives

    a. Common salt

    b. Sugar

    c. Dextrose

    d. Glucose

    e. Spices

    f. Vinegar or acetic acid

    g. Honey

    h. Edible vegetable oil

Addition of class I preservatives in any food is not restricted, unless otherwise provide in the rule.

Class II preservatives

a.   Benzoic acid including salts theirof

b.   Sulphurous acid including salts theirof

c.   [Nitrates of] nitrites of sodium or potassium

d.   Sorbic acid including its sodium, potassium and calcium salts

e.   Nicin

f.   Propionic acid including salts theirof

g.   Methyl or propyl para-hydroxy benzoate

h.   Sodium diacetate

i.   Sodium, potassium and calcium salts of lactic acid

Use of class II preservatives is restricted. They shall be added to only specified product and at a concentration not exceeding the proportion specified for the product

Use of more than one class II preservative is prohibited. No person shall use in or upon a food more than one class II preservative

32.2.1. Benzoic acid and its salt

Widely use as an antimicrobial agent. Benzoate is more effective against yeasts and bacteria than molds. Antimicrobial activity is achieved by inhibition in enzymatic system of microbial cells, affecting acetic acid metabolism, citric acid cycle and oxidative phosphorylation.

Antimicrobial activity is affected by pH of medium The maximum inhibition occurs at pH value of 2.5 to 4.0 and it decreases when pH rises above 4.5.

The food products preserved with the benzoate include fruit juices and drinks, salads, jams and jellies, pickles, dried fruits and preserves, ketch up and sauce, syrup, carbonated beverages, bakery items, salad dressings, margarine and other fat spreads, spices.

32.2.2. Sulphur dioxide and sulfites

Sulphur dioxide (SO_2) gas is one of the oldest antimicrobial agents. It is a colourless, non-flammable gaseous compound or liquid under pressure with a suffocating pungent odour. When dissolved in water of foods, it yields sulphurous acid and its ions, owing to its solubility in water.

Sulphite salts such as sodium sulphite, sodium bisulphite, potassium sulphite, potassium bisulphite, sodium metabisulphite, potassium metabisulphite used as preservatives. When dissolved in water, form sulphurous acid, bisulphite and ions. Sulphurous acid formed from these compounds is an active antimicrobial substance. The effectiveness of sulphurous acid is enhanced at low pH values. Antimicrobial activity of sulfites against yeasts, molds and bacteria is selective, with certain species being more sensitive to inhibition them others. Bacteria are generally more sensitive to inhibition than yeasts and molds. In addition to antimicrobial action, they are also used, to prevent enzymatic and non enzymatic changes as well as discoloration in some foods. Sulphur dioxide and sulphites are used in fruit products such as fruit juice concentrate, squashes, pickles and chutneys.

32.2.3. Sorbic acid and its salts

Sorbic acid and its salts (calcium, potassium or sodium salts) are effective antimicrobial agents against yeast and molds, as well as bacteria. They are less effective against bacteria. Sorbate has an upper pH limit for activity around 6.0-6.5. The food products preserved with sorbates are carbonated beverages, salad dressings, tomato products, jams, jellies, syrup, candy and chocolate syrup, cheese, sausages, smoked fish, fruit juices, grains, breads and cakes.

32.2.4. Propionic acid & its salts

Propionic acid & its salts (Ca & Na) are used most extensively in the prevention of mold growth and rope development in baked goods and for mold inhibition in many cheese foods and spreads. They are more effective against molds as compared to yeasts and bacteria. Propionates has an upper pH limit for activity around 5 to 6.

32.2.5. Lactic Acid & Its Salts

Lactic acid is formed during fermentation of lactose by lactic acid bacteria. Lactic acid & its salts are not very common & not easily available. It can be used in pickles (with acetic acid), fermented dough crispy biscuits, some beverages, dairy products & meat & meat products. Calcium lactate is used as a firming agent in pickles, fruits & vegetables. Na & K lactate are also recommended with sodium diacetate for control of food poisoning & othee bacteria in meat product.

32.2.6. Acetic Acid

Acetic acid has antimicrobial properties. The action tends to be static rather than cidal. It is more effective against bacteria & yeast then molds. A 5 to 10 % solution of acetic acid is known as Vinegar. Acetic acid in the form of vinegar is used in mayonnaise, pickles, sauce, pickled sausage etc.

32.2.7. Sodium Chloride (Common salt)

Antimicrobial action of NaCl arises from its lowering water activity (a_w) of the food product. This reduces available water in food to the extent which renders condition unfavorable for microbial growth. At higher concentration it has a pronounced bacteriostatic action. The 10 % NaCl inhibits the growth of most bacteria. Delaying action upon microorganisms- Creates dehydration of microbial cell—by osmosis—altering results into plasmosis of the cell. Reduction in solubility of oxygen in water decreases oxygen level in food—reduce growth of aerobic microorganisms. It is more effective against bacteria & mold compare to yeast.

One of the traditional method of food preservation. Mainly used to preserve pickles, meat & fish. Fish is usually salted by immersing in brine or by mixing with dry salt. High important as a preservative for cheese & table butter. Depending upon type of cheese salt content varied from 1 to 5 %. In table butter salt is added at a max concentration as 3 %.

32.2.8. Sucrose (Sugar)

More effective against bacteria & mold compared to yeast. Antimicrobial action of sucrose arises from, lowering water activity (aw) of the food product—reduce the available water in food to the extent which renders condition unfavourable for microbial growth. This creates dehydration of microbial cell—by osmosis results into plasmosis of the cells. The food products preserved with sugar are fruit products (jam, jellies, squash etc.), dairy products (sweetened condensed milk, sweets).

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