FOOD CHEMISTRY AND CHEMICAL ANALYSIS OF FOODS

(DFNL21B)

(MSC – F.N.S, PRACTICAL)

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Food Chemistry and Chemical Analysis of Foods
CONTENTS

Lesson – 1

WATER

1.0 Objective

After going through this lesson you will learn:

- The components of water
- The chemistry of water
- Types of water
- Water activity

Structure

1.1 Introduction

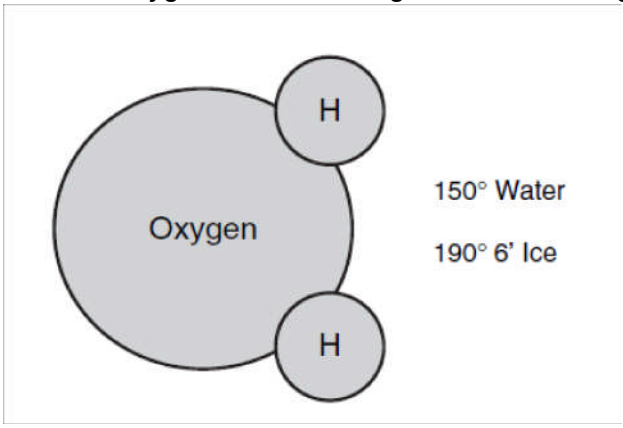
Water is abundant in all living things and consequently is in almost all foods, unless steps have been taken to remove it. It is essential for life, even though it contributes no calories to the diet. Water also greatly affects the texture of foods, as can be seen when comparing grapes and raisins (dried grapes), or fresh and wilted lettuce. It gives crisp texture or turgor to fruits and vegetables and also affects perception of the tenderness of meat. For some food products, such as potato chips, salt, or sugar, lack of water is an important aspect of their quality and keeping water out of such foods is important to maintain quality.

Almost all food processing techniques involve the use of water or modification of water in some form: freezing, drying, emulsification (trapping water in droplets), bread making, thickening of starch, and making pectin gels are a few examples. Further, because bacteria cannot grow without water, the water content has a significant effect on maintaining quality of the food. This explains why freezing, dehydration, or concentration of foods increases shelf life and inhibits bacterial growth. Water is important as a solvent or dispersing medium, dissolving small molecules to form true solutions and dispersing larger molecules to form colloidal solutions. Acids and bases ionize in water;

Water also is necessary for many enzyme-catalyzed and chemical reactions to occur, including hydrolysis of compounds such as sugars. It also is important as a heating and cooling medium and as a cleansing agent. Because water has so many functions that are important to a food scientist, it is important to be familiar with some of its unique properties. When modifying the water content of a food, it is necessary to understand these functions in order to predict the changes that are likely to occur during processing of such foods. Drinking water is available to the consumer in convenient bottled and aseptic containers in addition to the tap.

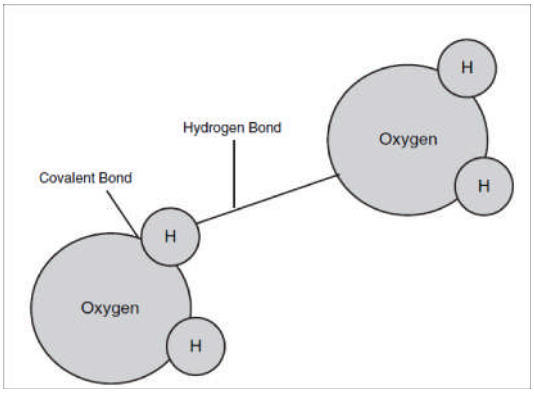
1.2 Chemistry of Water

Water contains strong covalent bonds that hold the two hydrogen atoms and one oxygen atom together. The bonds between oxygen and each hydrogen atom are polar bonds, having a 40% partial ionic character. This means that the outer-shell electrons are unequally shared between the oxygen and hydrogen atoms, the oxygen atom attracting them more strongly than each hydrogen atom.



Bond angle of water and ice

As a result, each hydrogen atom is slightly positively charged and each oxygen atom is slightly negatively charged. Therefore they are able to form hydrogen bonds. A hydrogen bond is a weak bond between polar compounds where a hydrogen atom of one molecule is attracted to an electronegative atom of another molecule. It is a weak bond relative to other types of chemical bonds such as covalent or ionic bonds, but it is very important because it usually occurs in large numbers, and therefore has a significant cumulative effect on the properties of the substance in which it is found. Water can form up to four hydrogen bonds (oxygen can hydrogen-bond with two hydrogen atoms).



Hydrogen and covalent bonds in water molecules

Liquid water has a smaller bond angle than ice, the molecules can be packed together more tightly, and so the coordination number, or in other words the average number of nearest neighbors is higher for water than for ice. The average distance between water molecules also is affected by temperature and increases with temperature as the molecules have more kinetic energy and can move around faster and farther at higher temperatures. Both of these affect the density of water.

Ice is less dense than water because the molecules have a smaller coordination number and cannot be packed together as tightly as water. Therefore, ice floats. As water freezes, its density decreases and its volume increases by about 9%. This is very significant when freezing foods with high water content. Containers and equipment must be designed to accommodate the volume increase when the product freezes; for example, molds for popsicles must allow room for expansion. This volume increase also contributes to the damage to the structure of soft fruits on freezing.

1.3 Types of water

Water is abundant in all living things, and consequently in almost all foods, unless steps have been taken to remove it. Most natural foods contain water up to 70% of their weight or greater unless they are dehydrated, and fruits and vegetables contain water up to 95% or greater. Water that can be extracted easily from foods by squeezing or cutting or pressing is known as ***free water***, whereas water that cannot be extracted easily is termed as ***bound water***.

Bound water usually is defined in terms of the ways it is measured; different methods of measurement give different values for bound water in a particular food. Many food constituents can bind or hold onto water molecules, such that they cannot be removed easily and they do not behave like liquid water. Some characteristics of bound water include:

- It is not free to act as a solvent for salts and sugars.
- It can be frozen only at very low temperatures (below freezing point of water).
- It exhibits essentially no vapour pressure.
- Its density is greater than that of free water.

Bound water has more structural bonding than liquid or free water; thus, it is unable to act as a solvent. As the vapour pressure is negligible, the molecules cannot escape as vapour; the molecules in bound water are more closely packed than in the liquid state, so the density is greater. An example of bound water is the water present in cacti or pine tree needles—the water cannot be squeezed or pressed out; extreme desert heat or a winter freeze does not negatively affect bound water and the vegetation remains alive. Even upon dehydration, food contains bound water. Water molecules bind to polar groups or ionic sites on molecules such as starches, pectins, and proteins. Water closest to these molecules is held most firmly; the subsequent water layers are held less firmly and are less ordered, until finally the structure of free water prevails. Water also may be entrapped in foods such as pectin gels, fruits, vegetables, and so on. Entrapped water is immobilized in capillaries or cells, but if released during cutting or damage, it flows freely. Entrapped water has properties of free water and no properties of bound water.

A promising new method is the use of face tension a in a capillary with radius *r* is subject to a pressure loss, the capillary pressure P_0 = 2σ/r, as evidenced by the rising of the liquid in the capillary. As a result, there is a reduction in vapor pressure in the capillary, which can be expressed by the Thomson equation,

$$\ln\frac{p}{p_o} = -\frac{2\sigma}{r}\cdot\frac{V}{RT}$$

where

p = vapor pressure of liquid

P_0 = capillary vapour pressure

a = surface tension

V = mole volume of liquid

R = gas constant

T = absolute temperature

In water-rich organic foods, such as meat and potatoes, the water is present in part in capillaries with a radius of 1 ìm or more. The pressure necessary to remove this water is small. It is evident that water from capillaries of 0.1 ìm or larger can easily drip out. Structural damage caused, for instance, by freezing can easily result in drip loss in these products. The fact that water serves as a solvent for many solutes such as salts and sugars is an additional factor in reducing the vapour pressure.

Radius (nm)	Activity (a)
0.5	0.116
1	0.340
2	0.583
5	0.806
10	0.898
20	0.948
50	0.979
100	0.989
1000	0.999

Capillary radius and water activity

The non freezable and Langmuir water are probably not exactly the same. The amount of free water in beef, pork, veal, and lamb varies from 30 to 50 percent of total moisture, depending on the kind of meat and the period of aging, a sharp drop in bound water occurs during the first day

after slaughter, and is followed by a gradual, slight increase. At the normal pH of meat there is a considerable reduction of bound water.

Radius	Pressure (kg/cm²)
0.1 ìm	14.84
1 ìm	1.484
10 ìm	0.148
0.1 mm	0.0148
1 mm	0.0015

Pressure required to press water from tissue at 20⁰C

1.4 Water Activity

In 1952, Scott came to the conclusion that the storage quality of food does not depend on the water content, but on water activity (aw), which is defined as follows:

$$a_w = P/P_0 = ERH/100$$

P = partial vapor pressure of food moisture at temperature T

P_0 = saturation vapor pressure of pure water at T

ERH = equilibrium relative humidity at T.

The relationship between water content and water activity is indicated by the sorption isotherm of a food. At a low water content (<50%), even minor changes in this parameter lead to major changes in water activity. As a rule, the position of the hysteresis loop changes when adsorption and desorption are repeated with the same sample. Decreased water activity retards the growth of microorganisms, slows enzyme catalyzed reactions (particularly involving hydrolases; cf. 2.2.2.1) and, lastly, retards non-enzymatic browning.

In contrast, the rate of lipid autoxidation increases in dried food systems. Foods with a_w values between 0.6 and 0.9 are known as “intermediate moisture foods” (IMF). These foods are largely protected against microbial spoilage. One of the options for decreasing water activity and thus improving the shelf life of food is to use additives with high water binding capacities (humectants).

Moisture Content	
Peas	16
casein	19
Starch (potato)	20
Glycerol	108
Sorbitol	67
saccharose	56
Sodium chloride	332

Moisture content of some food or food ingredients at a water activity of 0.8

1.4.1 Water Activity as an Indicator

Water activity is only of limited use as an indicator for the storage life of foods with a low water content, since water activity indicates a state that applies only to ideal, i.e., very dilute solutions that are at a thermodynamic equilibrium. However, foods with low water content are non-ideal systems whose meta stable (fresh) state should be preserved for as long as possible. During storage, such foods do not change thermodynamically, but according to kinetic principles. A new concept based on phase transition, which takes into account the change in physical properties of foods during contact between water and hydrophilic ingredients, is better suited to the prediction of storage life.

1.5 Summary

Water contains strong covalent bonds that hold the two hydrogen atoms and one oxygen atom together. Ice is less dense than water because the molecules have a smaller coordination number and cannot be packed together as tightly as water. Therefore, ice floats. As water freezes, its density decreases and its volume increases by about 9%. In water-rich organic foods, such as meat and potatoes, the water is present in part in capillaries with a radius of 1 ìm or more. Water that can be extracted easily from foods by squeezing or cutting or pressing is known as *free water*, whereas water that cannot be extracted easily is termed as *bound water*. Bound water usually is defined in terms of the ways it is measured; different methods of measurement give different values for bound water in a particular food. The non freezable and Langmuir water are probably not exactly the same. The amount of free water in beef, pork, veal, and lamb varies from 30 to 50 percent of total moisture, depending on the kind of meat and the period of aging, a sharp drop in bound water occurs.

The relationship between water content and water activity is indicated by the sorption isotherm of a food. At a low water content (<50%), even minor changes in this parameter lead to major changes in water activity. The pressure necessary to remove this water is small. Water activity is only of limited use as an indicator for the storage life of foods with a low water content, since water activity indicates a state that applies only to ideal, i.e., very dilute solutions that are at a thermodynamic equilibrium.

1.6 Self Assessment Questions

1. What are the various components of water?
2. What is the chemistry of water?
3. What are the types of water?
4. What is water activity?

1.7. Reference Books

1. H.D.Belitz, W.Grosch and P.Schieber. Food Chemistry 4th revised and extended edition 2009
2. John M. deMan, Principles of food chemistry, Third edition, 1999

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Lesson – 2

SOLUTIONS

2.0 Objective

After going through this lesson you will learn

- The differences between colloidal solutions, true solutions and suspensions
- The concept of osmosis and hydrogen ion concentration

Structure

2.1 Introduction

All protoplasm is in colloid form. Most of the biological fluids, notably blood, lymph, milk, bile, and digestive secretions are colloidal solutions. Moreover, the biomembranes may themselves be considered to be a manifestation of the colloidal state. Based on distinct properties, solutions can be classified into True Solution, Suspension and Colloid. This classification is necessary to understand concepts of colloidal solutions and distinguish it from rest of the types.

2.2 Types of Solutions

2.2.1 Colloidal Solutions

These represent a state of subdivision of matter. The matter finally divided, is uniformly distributed in a continuous medium. However, the dispersed particles are neither so large that they separate on standing, nor so small that they can be said to be in solution. This means that the colloidal state is an intermediate state between a suspension and a true solution.

A colloidal system is characterized by particles ranging in size from 1mì to 0.1 ì in diameter. They can be formed either by aggregation of small molecules like sodium chloride or by disintegration of large polymers. The particles are non-filterable and can be observed under the ultra microscope as illuminated discs engaged in a kind of random zig-zag motion. This type of movement is called Brownian movement. The smaller particles, the more rapid is their movement. The surrounding molecules of the dispersion medium continuously colloid with the colloidal particles. These collisions impart sufficient kinetic energy to the colloidal particles so as to enunciate a Brownian movement. This movement is, however considerably slower than that of the molecules of the medium and can therefore be observed under an ultra microscope.

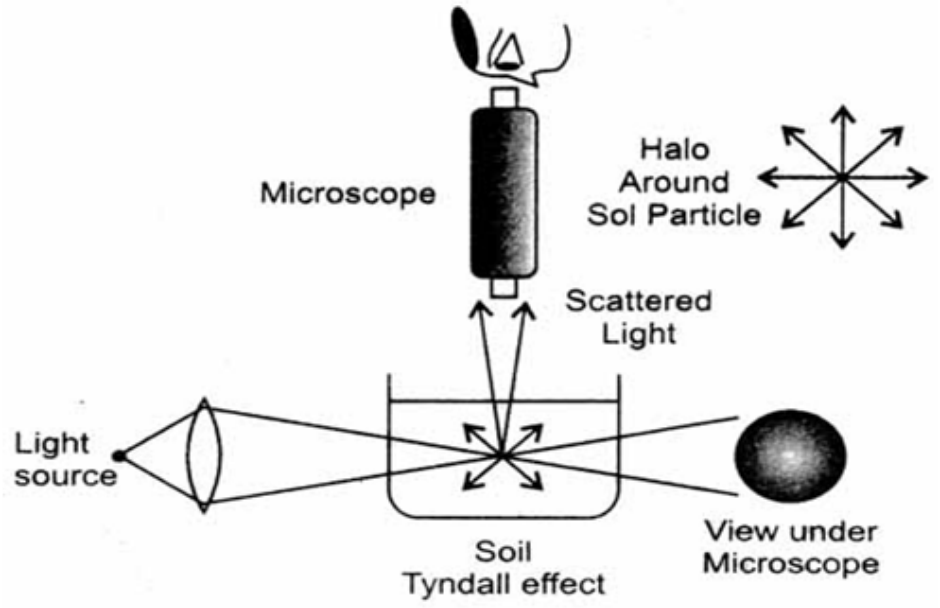
If the particles cross the upper size limit they will form the suspensions. In that case, the two phases separate on standing and the particles can be filtered off easily. An ordinary microscope is enough to observe the participants of the dispersed phase in a suspension. In contrast are the true solutions whose individual members are invisible even in the ultra microscope. Only an electron microscope can be used for this purpose as the size of the particles in such a system is always less than 1 mì.

Another characteristic property exhibited by colloidal system is the Tyndall effect. If a narrowly defined concentrated beam of light is allowed to pass through a colloidal system it appears as a white path. The same is not observed in a true solution. The above effect can be illustrated as follows. Consider a room with a small shutter on one of its walls. As long as the room is uniformly illuminated the air inside the room seems perfectly clear and transparent. But if the room is now darkened and concentrated beam of light allowed to enter through the shutter, we can see the dust particles in the path of the light clearly. This is due to scattering of light by dust particles. Minute dust particles in the path of the light clearly. This is due to scattering of light by dust particles. Minute dust particles affect the visible light waves to produce the phenomenon called diffraction. The same principle is followed by colloidal particles. True solutions do not respond to such a phenomenon because its particles are too small to scatter light.

The tyndall effect is utilized in the ultra microscope. In this instrument an intense light beam from an arc lamp is focused by the lenses of a compound microscope on the stage of another high-power microscope with its optical axis at right angles to the first one. Other light is excluded from the field. The individual sol particles scatter light and appear as bright discs against a dark background. Modern methods have made the use of ultraviolet light to extend the range of microscopic work. Its short wavelengths permit formation of focused images of objects as small a 10 mm in diameter. The images though not visible can be recorded photographically.

2.2.2. True Solutions

2.2.3. Suspensions



Tyndall effect

2.2.4 Classification of Colloids

Colloidal systems are composed two phase of matter. The dispersed phase, also called the discontinuous or internal phase is made up of colloidal particles while the dispersion medium, also called the continuous or external phase is made up of the solvent in which the dispersion takes place. Numerous types of combinations of these two phases are possible and it is difficult to classify colloids strictly. However, various attempts have been made and the classification of colloidal systems as two distinct types- the lyophilic colloids and the lyophobic colloids, based upon the interaction of phases has found wide acceptance.

2.2.4.1 Lyophilic Colloids

The word lyophilic stems from the Greek word phiols meaning loving (lyo means solvent). These are colloidal systems where there is strong attraction between the solvent and the particles. Numerous substances of biological interest such as proteins, nucleic acids and the starches fall in this category. Other significant compounds are the cholic acids, soaps, synthetic detergents, and the emulsifying agents. Lyophilic colloial solutions differ from true solutions only with respect to the size of the particles which leads to a change both in the properties as well as techniques of study of these systems.

A lyophilic colloidal system can take up different names depending on the nature of the dispersion medium. When it has water as the continuous phase it is called a hydrosol, while with alcohol as its dispersion medium it is called an alcosol. But the general term for this type of system is the emulsoid.

The emulsoids are easy to prepare and though they resemble the true solutions in many respects, the great disparity in size of the solute particles confer upon them some distinct physical

properties. Additional properties are conferred upon them because of their charged polyelectrolyte nature. One unique property of colloidal solution is the Donnan effect. It is unequal distribution of diffusible ions across a semipermeable membrane due to the concentration of colloidal particles on one side of the membrane.

Due to the high degree of salvation of the particles in an emulsoid, the precipitation or flocculation of these systems is difficult. They are stable and therefore are precipitated only by high concentration of electrolytes. Also, they exist in a state of true reversible equilibrium. To explain this further, the dispersed phase of lyophilic colloids when separated by evaporation or precipitation clump together in loosely packaged aggregates. These flocs can be reconstituted into the colloidal form easily by removing the electrolyte from the medium and by mixing.

The hydrophilic colloids show a higher viscosity than that of the medium. It has also been found that the viscosity increases with concentration. Two reasons are attributed to these observations.

- The hydrophilic colloids have strong attraction for water. This extensive salvation of the particle immobilizes the bound water and resists its freedom of movement thus increasing viscosity.
- With higher concentration of solute, there is more trapping of solvent. Also the colloidal particles try to orient themselves in a lattice like structure. This systematic arrangement affects the flow of the solvent as the solvent now has to find gaps in this structure of escape or it has to break down this structure to enable its flow.

The optical properties of lyophilic systems are also different. Tyndall effect for these systems is minimum. Besides the size of the particles, the difference in refractive indices between the dispersed phase and the dispersion medium also affect tyndallization. In an emulsoid this difference is not appreciable because of the high salvation character of this system. Therefore the Tyndall effect is not so prominent.

2.2.4.2 Lyophobic Colloids

The lyophobic colloids derive their name from the Greek work phobe (meaning fearing or hating) and thus mean ‘solvent-hating’. But we cannot call these systems ’solvent hating’ in the strict sense because if it was so, no dispersion would be formed. If the solute molecule completely repelled the dispersion medium it would have remained dry. No wettng and therefore no dispersion was possible. However, lyophobic systems do form dispersed systems in which the molecules remain detached from the solvent though not in complete isolation. This is one of the reasons of their instability.

The lyophobic systems are also called suspensoids. They easily precipitate out forming irreversible flocculates. But if they are left undisturbed, despite their intrinsic instability they remain uncahged for long periods.

2.2.2 True Solutions

True Solution is a homogeneous mixture of two or more substances in which substance dissolved (solute) in solvent has the particle size of less than 10-9 m or 1 nm. Simple solution of sugar in water is an example of true solution. Particles of true solution cannot be filtered through filter paper and are not visible to naked eye.

2.2.4 Suspensions

Suspension is a heterogeneous mixture in which particle size of one or more components is greater than 1000nm.When mud is dissolved in water and stirred vigorously, particles of mud are distributed evenly in water. After some time, the particles of this solution settle under water due to influence of gravity. This solution is an example of Suspension (see picture below). Contrary to True Solution,particles of suspension are big enough to be seen with naked eye.

Property	True solution	Colloidal solutions	Suspension
Size of the particles	< 1nm	1– 1000nm	>1000nm
Nature	Homogeneous	Heterogeneous	Heterogeneous
Filterability (Diffusion through parchment paper)	Particles of true Solution diffuse rapidly through filter paper as well as parchment paper.	Colloidal particles pass through filter paper but not through parchment paper	Suspension particles do not pass through filter paper and parchment paper.
Visibility	Particles of true solution are not visible to naked eye	Colloidal particles are not seen to naked eye but can be studied through ultra microscope.	Suspension particles are big enough to be seen by naked eye.
Tyndall effect	True solution does not show Tyndall effect	Colloids shows Tyndall effect	Suspension may or may not show Tyndall effect
Appearance	Transparent	Translucent	Opaque

Distinction between each type of solution with respect to different properties

2.3 Osmosis

It is one of the major exchange processes which is beneficial to the living body and therefore has to be regulated carefully. It is not only the composition of the various ions in the body fluids but also their content which is important for a properly functioning individual. Osmosis takes care of both of these. By definition it is the flow of solvent from a dilute to a concentrated solution, across a semi permeable membrane till the system attains equilibrium. The semi permeable nature varies for membrane to membrane. For some, it is semi permeable to small ions and molecules but not big colloidal particles, for others it may be differentially permeable to certain ions, excluding others, while some may be totally non specific. Biological membranes, though, are mostly specific.

They have a vast range of transport systems embedded within them which will carefully ‘sniff’ the metabolite to be transported while it waits outside and only allow on kind to enter, that

which is needed. Similarly the one to go out also has to face scrutiny. This flux of metabolites is associated with the movement of solvents. Some of these movements require energy and thus are termed active while others just move on across the gradient and therefore called passive. Osmosis belongs to the later category. It is concerned with the spontaneous flow of solvent only. We can demonstrate this by a simple experiment. A glass tube open at both ends is chosen and at one end is tied an animal membrane in the form of a sack. Now the tube is half filled with concentrated sugar solution and then dipped in a beaker of water. After some time we observed the liquid in the tube has risen. The level of the liquid rises until the hydrostatic pressure so developed is sufficient to stop the flow of solvent, or osmosis, into the tube. This hydrostatic pressure as a result of osmosis is called the osmotic pressure of the solution and is defined as the excess pressure that must be applied to the solution to prevent the passage of the solvent in to it through the semi permeable membrane separation the two solutions.

2.4 Hydrogen Ion Concentration

The ion product of water, *K*w, is the basis for the pH scale. It is a convenient means of designating the concentration of H^+ (and thus of OH^-) in any aqueous solution in the range between 1.0 M H^+ and 1.0 M OH^-. The term pH is defined by the expression

$$pH = \log \frac{1}{[H^+]} = -\log [H^+]$$

The symbol p denotes "negative logarithm of." For a precisely neutral solution at 25 ^{0}C, in which the concentration of hydrogen ions is 1.0 x 10^{-7} M, the pH can be calculated as follows:

$$pH = \log \frac{1}{1.0 \times 10^{-7}} = \log (1.0 \times 10^7)$$
$$= \log 1.0 + \log 10^7 = 0 + 7 = 7$$

The value of 7 for the pH of a precisely neutral solution is not an arbitrarily chosen figure; it is derived from the absolute value of the ion product of water at 25 ^{0}C, which by convenient coincidence is a round number. Solutions having a pH greater than 7 are alkaline or basic; the concentration of OH^- is greater than that of H^+. Conversely, solutions having a pH less than 7 are acidic. Note that the pH scale is logarithmic, not arithmetic.

To say that two solutions differ in pH by 1 pH unit means that one solution has ten times the H^+ concentration of the other, but it does not tell us the absolute magnitude of the difference. A cola drink (pH 3.0) or red wine (pH 3.7) has an H^+ concentration approximately 10,000 times that of blood (pH 7.4). The pH of an aqueous solution can be approximately measured using various indicator dyes, including litmus, phenolphthalein, and phenol red, which undergo color changes as a proton dissociates from the dye molecule. Accurate determinations of pH in the chemical or clinical laboratory are made with a glass electrode that is selectively sensitive to H^+ concentration but insensitive to Na^+, K+, and other cations.

In a pH meter the signal from such an electrode is amplified and compared with the signal generated by a solution of accurately known pH. Measurement of pH is one of the most important and frequently used procedures in biochemistry. The pH affects the structure and activity of biological macromolecules; for example, the catalytic activity of enzymes is strongly dependent on pH.

Measurements of the pH of blood and urine are commonly used in medical diagnoses. The pH of the blood plasma of people with severe, uncontrolled diabetes, for example, is often below the normal value of 7.4; this condition is called acidosis. In certain other disease states the pH of the blood is higher than normal, the condition of alkalosis.

2.5 Summary

A colloidal system is characterized by particles ranging in size from 1mì to 0.1 ì in diameter. They can be formed either by aggregation of small molecules like sodium chloride or by disintegration of large polymers. The classification of colloidal systems as two distinct types- the lyophilic colloids and the lyophobic colloids, based upon the interaction of phases has found wide acceptance. True Solution is a homogeneous mixture of two or more substances in which substance dissolved (solute) in solvent has the particle size of less than 10-9 m or 1 nm. Suspension is a heterogeneous mixture in which particle size of one or more components is greater than 1000nm.When mud is dissolved in water and stirred vigorously, particles of mud are distributed evenly in water. After some time, the particles of this solution settle under water due to influence of gravity.

Osmosis is the flow of solvent from a dilute to a concentrated solution, across a semi permeable membrane till the system attains equilibrium. Some of these movements require energy and thus are termed active while others just move on across the gradient and therefore called passive. Osmosis belongs to the latter category. The pH affects the structure and activity of biological macromolecules; for example, the catalytic activity of enzymes is strongly dependent on pH. Measurements of the pH of blood and urine are commonly used in medical diagnoses.

2.6 Self Assessment Questions

1. What are the differences between true solutions, colloidal solutions and suspensions?
2. Explain about the tyndall effect.
3. What is the biological significance of osmosis?
4. What is hydrogen ion concentration?

2.7 Reference Books:

1. H.D.Belitz, W.Grosch and P.Schieber. Food Chemistry 4th revised and extended edition 2009

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LESSON - 3

PHYSICOCHEMICAL PRINCIPLES WITH SPECIAL REFERENCE TO FOODS

3.0 Objective

After going through this lesson you will learn:

- Various rheological methods
- Colour analysis of foods
- Thermal analysis of foods

Structure

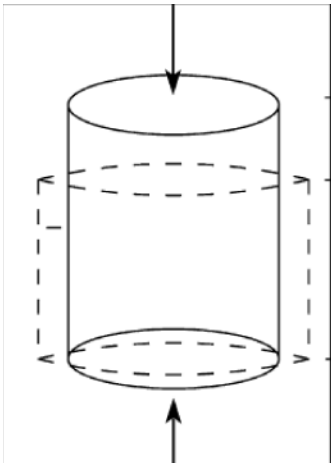
3.1 Introduction

Rheology is concerned with stress-strain relations of materials that show a behavior intermediate between those of solids and liquids. Stress can be compressive, tensile, or shear.

The passage of time does not itself cause changes in materials. Time is, however, introduced often in measuring rates of changes of forces and deformations. Chemical changes in food stuffs often occur in time and may be studied by rheological methods. Temperature is also, important and often appears in rheological equations.

3.2 Strain

It is measured by deformation. All deformations are divided into elastic deformations and flow. An elastic deformation is one that can be recovered, irrespective of whether the recovery is spontaneous or not. Elastic deformations may be Hookean or non-Hookean; in the latter strain may increase more or less rapidly than stress. Non ideal deformations may be completely recoverable, and the strain may vary more rapidly than, proportionally to, or less rapidly than stress. The incompletely recoverable deformations are linked with flow, and also lead to plasto elastic and plasto inelastic groups. Plastic deformations may be Newtonian or non-Newtonian; the latter is subdivided into visco elastic and visco inelastic groups.



Normal stress in a cylinder

3.3 Texture

It can be regarded as a manifestation of the rheological properties of a food. It is an important attribute in that it affects processing and handling, influences food habits, and affects shelf-life and conscious of food texture and in certain foods texture may be even more important than flavour.

Measurement of the rheological properties of foods can be based on either the analytical or the integral approach. In the first, the properties of a material are related to such simple systems as Newtonian fluids or Hookean solids. When the material approximates any such system, the appropriate equations are used with a suitable correction. For a material that does not exhibit perfect elastic or true viscous behavior, the hypothesis is made that exhibit perfect elastic or true viscous behavior, the hypothesis is made that the material consists of two or more parts, and that the effects are additive.

In the integral approach, a simple and initially empirical relation between stress, strain, and time is sought. This approach is often dictated by the fact that its results correlate better with sensory evaluation than descriptions based on rheological models or simple dimensional terms. Foods seldom have simple rheological properties. In addition, most rheological measurements

refer to the arbitrary conditions imposed by a particular instrument. What we are generally measuring is not a pure rheological parameter, but the way in which the properties vary under some standardized system of applied forces.

Yet, the relationship between stress-strain systems and their time derivatives are often expressed in terms of models- a pictorial presentation of the analytical approach. The models sometimes have no more than a symbolic significance; in other cases they throw light on existing configurations and are a very valuable rheological tool.

3.4 Rheological Models

These are useful because they represent, in an easily comprehensible way, the mechanical behavior of materials and facilitate mathematical description of their behavior. The four basic components include a dashpot (piston sliding in a cylinder filled with oil) depiction Newtonian viscosity (N), the spring depicting the Hookean elasticity element (H), the spring clip or St. Venant body (St. V), and the shear pin (SP). The dotted lines above the deformation-time plots represent the timing of loading and unloading. Upon loading, the spring (H) lengthens immediately by an amount dependent on the load, whereas in the dash pot (N) it is the speed of movement that is proportional to the load. When the dashpot (N) is unloaded, the deformation remains; when the spring is unloaded, there is instantaneous and complete recovery.

The St.Venant element (St. V) is shown by a friction weight. It describes ideal plasticity, that is, an irrecoverable deformation caused at a specific stress above the yield value. Below this, no deformation takes place; above it, deformation continues at a constant rate as long as the yield stress persists. The shear pin (SP) allows presentation of the rupture of an element under a specific stress.

A dashpot and a spring combined in a series (H-N) give a model of a Maxwell body. When the spring and dashpot are combined in parallel (H/N), the model is referred to as the Kelvin or Voigt type. When applied, the load is gradually transferred from the dashpot to the spring, which eventually tends to hold the load entirely. This causes the deformation-time curve to level out asymptotically. On unloading, there is slow but complete recovery as the spring recovers against the friction of the dashpot.

3.5 Rheological Instruments

Viscometers are the main time-measuring instruments. Energy-measuring instruments determine work or energy and include recording dough mixers or meat grinder. If a force-distance curve is drawn during test, the area under the force-distance curve is a measure of work. For ratio-measuring methods, at least two measurements of the same variable must be taken. Thus, cohesiveness can be computed from the ration of the work done during the first and second bites of food.

Instruments in the multiple-measuring group can measure various forces, distances, areas, etc., and record the results. The instruments can be used for a large number of products. They include the **instron.** Generally, a special test cell for each type of product is necessary for meaningful evaluation. Multiple-variable instruments have more than on uncontrolled variable, but only one variable is measured. Although sometimes these instruments correlate well with sensory evaluation, it is difficult to evaluate the results in terms of fundamental rheological parameters or to relate the measurements with data from other instruments.

Instron model 1122 testing equipment with punch probe

Chemical analyses do not measure texture directly, but are often highly correlated with physical texture measurements or subjective panel tests. A typical chemical determination of this kind is the content of alcohol-insoluble solids, an accepted index of green peas maturity. In addition, there exist objective methods that are highly correlated with texture or consistency of a food, and do not fit any of the above groups. This group of miscellaneous methods includes various refractometric, polarimetric, electrical and sound testing devices.

3.6 Measuring the Components of Food Texture

The basic measurement in a hardness determination involves the load deformation relationship. Cohesiveness may be measure as the rate at which the material disintegrates under mechanical action. Tensile strength is a manifestation of cohesiveness. Cohesiveness is usually tested in terms of the secondary parameters brittleness, chewiness and gumminess. Brittleness, crunchiness and crumbliness, which can be placed on a continuum can be measured as the ease with which the material yields under an increasing compression load; the smaller the deformation under a given load, the lower the cohesiveness and the greater the “snappability” of the product. Tenderness, chewiness, and toughness are measured in terms of the energy required to masticate a solid food. They are the characteristics most difficult to measure precisely, because mastication involves compressing, shearing, piercing, grinding, tearing and cutting, along with adequate lubrication by saliva at body temperatures.

Gumminess is characteristic of semisolid foods with a low degree of hardness and a high degree of cohesiveness. The rate at which a food returns to its original condition after removal of a deforming force is an index of the food’s elasticity. Adhesiveness is measured in terms of the work required to overcome the attractive forces between the surface of a food and the surface of other materials with which the food comes into contact.

The frequency spectra and the amplitude-time characteristics of sounds produced by biting crisp foods cover a wide range and show irregular variations in loudness with time. These workers have proposed a model system, involving a generalized cellular structure, in which the sounds produced as crisp cellular foods are crushed result from the rupture of single cells or cell walls. Foods vary widely in the recognizability of their crushing sounds. The recongnizability does not depend on the familiarity or class of a food. The relationship between oral and auditory judgments of crispness, crunchiness and hardness are highly correlated. It was also proposed that vibrations produced by fracturing crisp foods may be the basis of perception of crispness in a variety of foods.

3.7 Colour Analysis

Color can be defined as the sensation that is experienced by an individual when radiant energy within the visible spectrum (380–770 nm) falls upon the retina of the eye, and a colorant is a pigment that is used to color a product. For the phenomenon of color to occur there must be

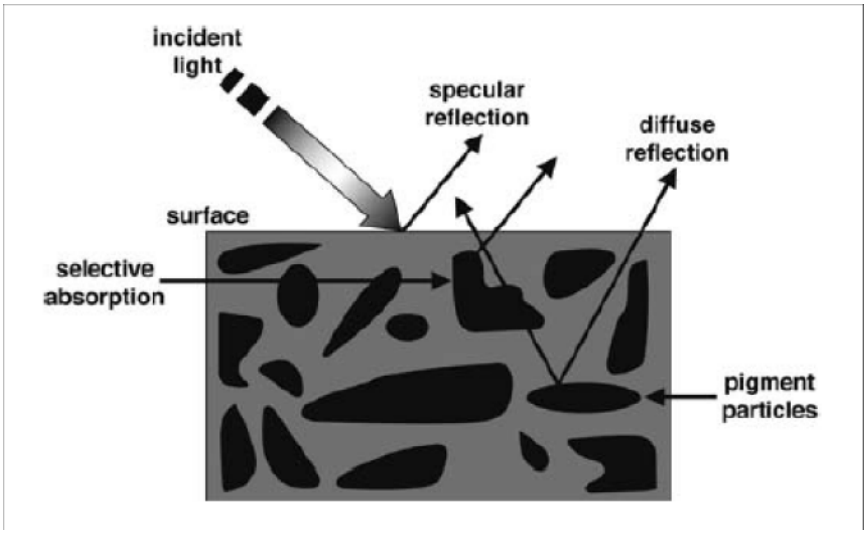
- a colored object,
- light in the visible region of the spectrum, and
- an observer.

All three of these factors must be taken into account when assessing and measuring color. When white light strikes an object it can be absorbed, reflected, and/or scattered. Selective absorption of certain wavelengths of light is the primary basis for the color of an object. Color, as seen by the eye, is an interpretation by the brain of the character of light coming from an object. Colorimetry is the science of color measurement.

3.8 Practical Considerations in Colour Measurement

3.8.1 Interaction of Light with Sample

When a sample is illuminated with light a number of things occur that are illustrated in fig. Light for which the angle of reflection is equal to the angle of incidence is described as specular light. Smooth polished surfaces will appear glossy because of the high degree of specular reflection. Rough surfaces will have a great deal of diffuse reflection and will have a dull or matte appearance. Selective absorption of light will result in the appearance of color. Opaque samples will reflect light.



Interaction of light with an object

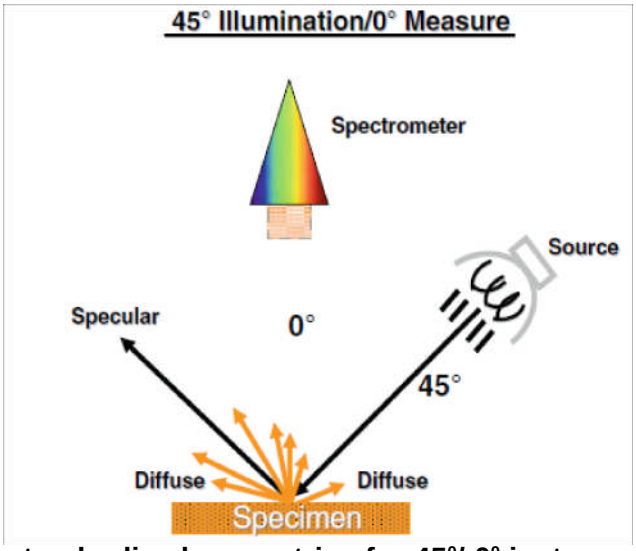
Transparent samples will primarily transmit light, and translucent samples will both reflect and transmit light. Ideal samples for colour measurement will be flat, smooth, uniform, matte, and either opaque or transparent. A brick of colored cheddar cheese is one of the few food examples that come close to having those characteristics.

3.9 Instrument Choice

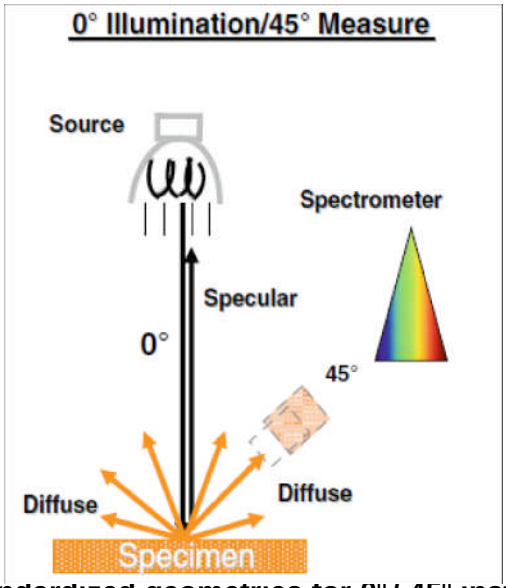
Instrument geometry refers to the arrangement of light source, sample placement, and detector. The CIE (international commission on illumination) recognizes the following instrument

geometries: $45^0/0^0$ where the specimen is illuminated at 45^0 and measured at 0^0, and the inverse, $0^0/45^0$ where the specimen is illuminated at 0^0 and measured at 45^0. Diffuse reflectance is measured since specular light is excluded. These are illustrated in figures given below.

Diffuse sphere geometry is the third type where a white-coated sphere is used to illuminate a sample. With some sphere geometry instruments, measurements can either include or exclude specular reflectance. These instruments are versatile in that they can measure in transmission for transparent samples and in reflectance for opaque samples. Some can also measure the amount of light scattering, turbidity or haze in liquid samples, and the amount of gloss in solid samples. Instruments with $45^0/0^0$ and $0^0/45^0$ geometries can only measure reflectance.



CIE standardized geometries for $45^0/ 0^0$ instrument



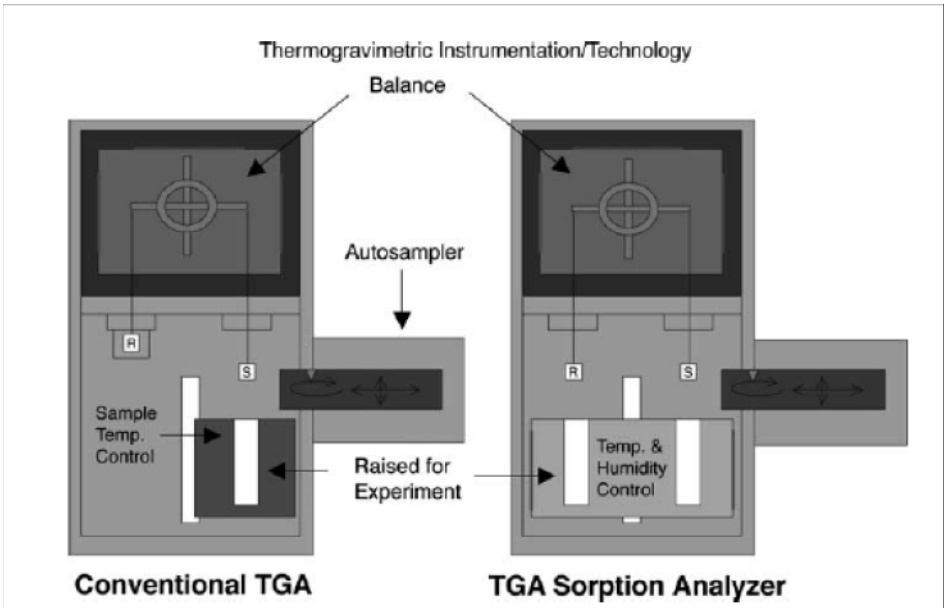
CIE standardized geometries for $0^0/ 45^0$ instrument

3.10 Thermal Analysis

Thermal analysis is a term used to describe a broad range of analytical techniques that measure physical and chemical properties as a function of temperature, time, and atmosphere (inert or oxidizing gas, pressure, and relative humidity). Depending on the technique, test temperatures can range from "180 to 1000^0 C or more, allowing investigation into a range of applications, including low temperature stability and processing (e.g., freezing and freeze-drying) to high temperature processing and cooking (e.g., extrusion, spray drying, and frying).

3.11 Thermogravimetric Analysis (TGA)

It should be the first thermal analysis technique used to characterize a new material. TGA provides information about the composition (number of components) of the material and its thermal or oxidative stability (decomposition in inert and oxidizing atmospheres, respectively). TGA instruments use a specially designed and very sensitive analytical balance to measure weight changes as the sample is typically heated from room temperature to a 1000^0 C or more. A thermocouple is located close to the sample to continuously record the temperature as weight changes occur. The heated sample chamber is typically purged with an inert gas, such as nitrogen or helium; however, air or oxygen can be used when measuring oxidative stability. Most weight changes are weight losses due to volatilization or decomposition, but weight gain is observed during early stages of oxidation. A specialized version of TGA is designed with humidity control so that the rate of moisture sorption (both absorption and desorption) can be measured as a function of time, temperature, and relative humidity.



Thermogravimetric analysis instrument

Common Measurements

TGA experiments are primarily heating experiments; however, isothermal (constant temperature) conditions can be used to determine drying rates or follow weight changes at processing/cooking temperatures. The most common measurements include the following:

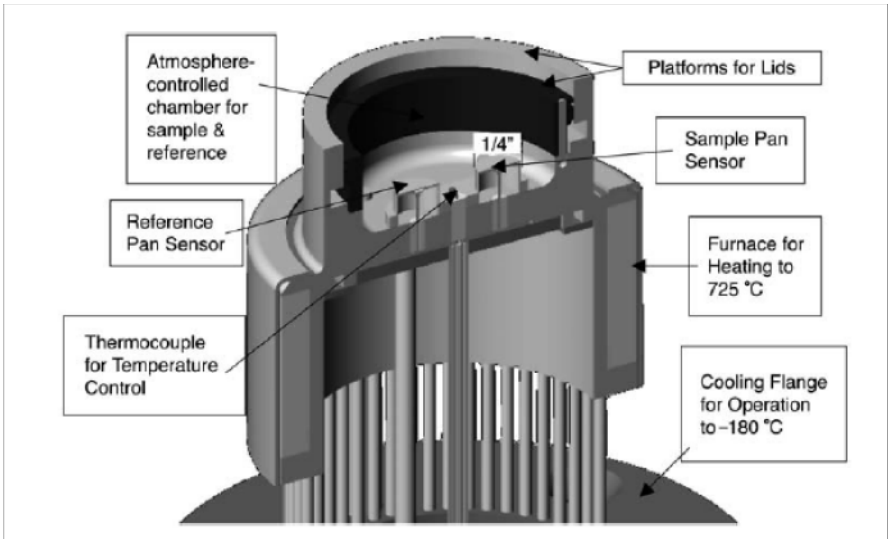
- Temperature of weight change
- Free (or bulk) moisture content “Bound” or associated water content (part of the structure)
- Composition (multiple components)
- Decomposition temperature;

In reality, there is no such thing as a decomposition temperature. Decomposition is a kinetic process, which means that it is a function of both time and temperature. Therefore, the temperature of weight loss due to decomposition increases if the heating rate is increased.

3.12 Differential Scanning Calorimetry (DSC)

It is the most frequently used thermal analysis technique and probably accounts for 70% of all thermal analysis measurements. Since every change in structure (transition) either absorbs or releases heat, DSC is the universal detector for measuring structure. The only limitation is the sensitivity of the instrument, which is its ability to detect small transitions or very slow kinetic processes where the rate of heat flow is similar to or less than the signal noise of the instrument.

- DSC measures the *sum* of all heat flows within the calorimeter. It is sometimes difficult to interpret data because of overlapping events (multiple transitions occurring at the same time and temperature).
- Most measurements involve heating the sample to higher temperatures. As temperature increases, mobility increases and this permits the structure to change in ways that are not always obvious. The measured structure may not be the original structure at the start of the experiment.
- DSC uses a single heating rate. However, higher heating rates provide better sensitivity, while lower heating rates provide better resolution. Therefore, it is not possible to optimize both sensitivity and resolution in a single DSC experiment.
- DSC cannot measure heat capacity under isothermal conditions. Therefore, DSC cannot use heat capacity as a way to follow changes in structure at constant temperature.



Cross-sectional view of a heat flux DSC

3.13 Summary

Rheological testing is simple in that it only requires the measurement of force, deformation, and time. To convert these measurements into fundamental physics based rheological properties requires an understanding of the material and testing method. Materials should be homogeneous and isotropic – true for most fluid foods and many solid foods. Fundamental rheological properties are determined based on knowledge of the stress or strain applied to the sample and the geometry of the testing fixture. Once rheological properties are determined they can be described by physical or mathematical models to gain a more complete understanding of the rheological properties.

The advantage of determining fundamental, rather than empirical, rheological properties is the use of common units, independent of the specific instrument, to determine the rheological property. This approach not only allows for comparison among values determined on different instruments, but it also permits a comparison of the flow of honey with the flow of paint. Through rheological methods, food scientists have the ability to relate theoretical and experimental information from a range of disciplines, including polymer chemistry and materials sciences, to gain a greater understanding of the quality and behavior of food materials.

Color is three dimensional, and any color-ordering or color-measuring system needs to address that fact. The Munsell system is a visual system that designates color in terms of hue, value, and chroma. Each of these dimensions has equivalent visual spacing, which is advantageous. The physiology of color vision has been long understood, and it provided the necessary background information for development of the CIE tristimulus system. Standardization of illuminants and experiments using humans with normal color version was necessary to develop colour-matching functions that corresponded to the color sensitivity of the human eye.

Thermal analysis is a series of laboratory techniques that measures physical and chemical properties of materials as a function of temperature and time. In a thermal analysis experiment, temperature is typically either held constant (isothermal) or programmed to increase or decrease at a linear rate. Since temperature and time are controlled in all food preparation processes, thermal analysis instruments can simulate these processes on a very small scale (milligrams) and measure the response of the material. The most frequently used techniques include DSC and TGA.

3.14 Self Assessment Questions

1. What is rheology of food?
2. What are the various rheological methods?
3. Why the colour analysis is important for foods?
4. What are the various practical considerations in colour measurement?
5. Explain about thermal analysis of foods?
6. Write the following
    a. Differential scanning calorimetry
    b. Thermogravimetric analysis

3.15 Reference Books

1. S. Suzanne Nielsen, Food analysis, 4th edition, 2010
2. Upadhyay and Upadhyay, Biophysical chemistry, 2006
3. Clifton.E.Meloan, food analysis 3rd edition (theory and practice)

Dr. P. KIRANMAYI

Lesson - 4

STARCH CHEMISTRY

4.0 Objective

After going through this lesson you will learn:

- Types of starches
- Chemistry of starch
- Factors effecting properties of starch
- Degree of retrogradation of starch

Structure

4.1 Introduction

Starch is the reserve carbohydrate of plants and occurs as granules in the cell in plastids, separated from the cytoplasm. Under the microscope the plastids in some plant tissues can be seen filled with granules and when the cells are ruptured, the granules stream out. The size and

shape of starch granules is characteristic of their origin, and anyone trained can readily identify the origin of starch granules with a microscope.

Most starch granules contain both amylose and amylopectin molecules. Waxy or glutinous starch from corn and other cereals contain little or no amylose, while a sugary mutant corn and some of the legumes contain amylose in greater abundance than amylopectin. Amylopectin is usually the more plentiful type of starch.

4.2 Types of Starches

Among food carbohydrates, starch occupies a unique position. It is the major carbohydrate storage material in many higher plants and is considered the second largest natural biopolymer next to cellulose. Starch is deposited in plant organs in the form of granules that are relatively dense, insoluble in cold water, and range from 1 to 100 ì m in size depending on the plant species. Starch contributes to the physicochemical properties of food products made from cereals, tubers, roots, legumes, and fruits. It is the basic source of energy for the majority of the world's population. In human nutrition, starch plays a major part in supplying the metabolic energy that enables the body to perform its different functions. Unlike some carbohydrates and digestible starches, resistant starch resists enzymatic hydrolysis in the upper gastrointestinal tract, thus resulting in little or no direct glucose absorption. In addition, resistant starch causes increased microbial fermentation in the large intestine to produce short-chain fatty acids, a similar physiological effect to dietary fiber.

Some fresh plant crops, such as corn, potato, and sweet potato, contain about 15% or more starch. Nuts such as chestnuts contain up to 33% starch. Starch also exists in the stem-pith (e.g., sago) and fruits (e.g., banana). The starch content increases with the degree of refinement in milled products; it is about 70% in white wheat flour compared to about 60% in whole grain. The increase in starch is accompanied by a parallel decrease in cellulose and hemicellulose. Starch is synthesized in the plastid compartment of plant cell. It is accumulated during the day in plant leaf cells and broken down at night to achieve a more or less constant supply of sucrose to the non photosynthetic tissues. Starch is formed by a complex biological pathway involving photosynthesis.

The starches vary in their thickening power. Wheat starches gelatinize earlier compared to rice, sorghum or corn starch. Corn or sorghum starch give opaque pastes of higher viscosity than wheat starch at the same concentration. Waxy corn starch does not form a get and remains clear because of lack of amylase. Flour that has been browned has less thickening power as some of the starch molecules had been converted to dextrins.

Industry	**Use of starch/ modified starch**
Food	Viscosity modifier, edible film, glazing agent
Adhesive	Binding
Paper and board	Binding, sizing, coating
Textile	Sizing, finishing and printing
pharmaceuticals	Diluents, binder, drug delivery
Detergent	Viscosity modifier
Agrochemical	Mulches, pesticide delivery, seed coating
Plastics	Food packaging, biodegradable filler
Cosmetics	Face and talcum powders
Purification	Flocculant
Medical	Plasma extender/replacers, transplant organ preservation

Industrial uses of starch

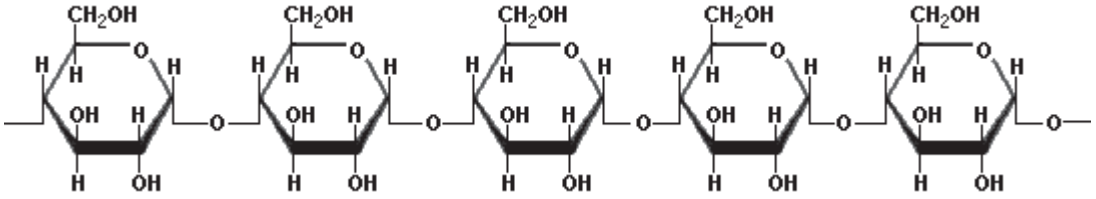
4.3 Starch Chemistry

Most starches are composed of a mixture of two molecular entities (polysaccharides), a linear fraction, amylose, and a highly branched fraction, amylopectin. The content of amylose is between 15 and 25% for most starches. The ratio of amylose and amylopectin in starch varies from one starch to another. The two polysaccharides are homoglucans with only two types of chain linkages, an á-(1→4) of the main chain an á-(1→6) of the branch chains. Although starch consists mainly of a homopolymer of á-D-glucopyranosyl units, it is one of nature's most complex materials. In some mutant species, starch granules may contain nearly 100% amylopectin. In addition to amylose and amylopectin, granules also contain some minor components such as proteins, lipids, inorganic substances, and nonstarch polysaccharides.

4.3.1 Amylose

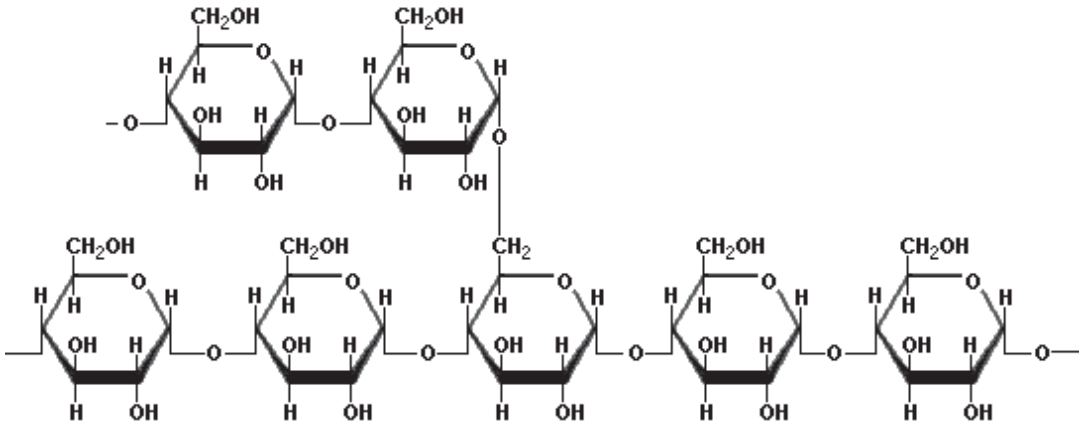
Physicochemical Properties

The abundance of hydroxyl groups along the amylose molecules imparts hydrophilic properties to the polymer, giving it an affinity for moisture. Because of their linear nature, mobility, and the presence of many hydroxyl groups along the polymer chains, amylose molecules have a tendency to orient themselves in a parallel fashion and approach each other closely enough to permit hydrogen bonding between adjacent chains. As a result, the affinity of the polymer for water is reduced and the solution becomes opaque. The interaction of amylose and iodine results in formation of complexes with characteristic color.



Amylose

4.3.2 Amylopectin



Amylopectin

Physicochemical Properties

The large size and the branched nature of amylopectin reduce its mobility in solution and eliminate the possibility of significant levels of inter chain hydrogen bonding. On average, amylopectin has one branch point every 20 to 25 residues. The branch points are not randomly located. The amylopectin chains can be classified into three types according to their length and branching points. The shortest A chains carry no branch points. The B chains are branched by A chain or other B chains (e.g., B1, B2, and B3 The C chain carries B chains but contains the sole reducing terminal residue. amylopectin are the crystalline regions present in the granules.

Another unique feature of amylopectin is the presence of covalently linked phosphate monoesters. They can be linked to either the C3 or C6 position of the glucose monomers, and occur to a greater extent in starch from tuberous species, especially potato starch.

4.4 Factors effecting properties of starch

4.4.1 Gelatinization

Starch granules do not dissolve readily in cold water but they will form a temporary suspension with the starch tending to settle out as soon as the mixture is allowed to stand. When heated with water the granules

a. Swell
b. The viscosity increases until a peak thickness is reached and
c. The translucency of the mixture also increases,

The term gelatinization is used in general to describe these changes. The changes appear to be gradual over a temperature range during gelatinization and the change transforms the temporary suspension in to a more permanent one. The swelling of starch particularly amylose resulting in the formation of a gel with water is believed to occur through the binding of water in starch the amylose and amylopectin molecules are loosly bound together by hydrogen bonds of the hydroxyls. The hydrogen on the hydroxyl on one molecule is attracted by the negative charge of the oxygen of a hydroxyl on other molecule and this attraction forms a weak link between the molecules.

As the temperature increases of the starch water mixture, the hydrogen bonding decreases for both the starch-starch bonds and water-water bonds and the size of the particles diminishes. Increasing water molecules begin to penetrate freely between the starch molecules when their kinetic energy becomes great enough to overcome the attraction between the starch molecules. two starch molecules which where originally bound together are know two starch molecules with water in between. The sticking together of granules is the result of molecules from adjacent granules becoming attracted and enmeshed in one and other. The change brought about by hot water and starch are irreversible.

Gelatinization Temperature

A starch mixture will start to thicken somewhere between 74 - 88^{0}C but complete gelatinization will not occur until the mixture is close to or at the boiling point. This will vary with the type of starch and size of the grain. Larger grains swell at a lower temperature. Gelatinization is complete for root starches such as potato at lower temperature because of the larger size of the

granules. In any case, swelling is usually complete at a temperature of 88 – 92^{0}C. Depending on the variety and storage conditions gelatinization temperatures vary.

The increase in the viscosity of the heated starch mixture is caused by the action of the enlarged starch granules bumping against each other. Trapping the water and inhibiting its free flow. Ones a starch mixture as reached the temperature at which gelatinization takes place, the mixture need only beheld at that temperature until the flavor of the uncooked starch has disappeared.

Completely gelatinized starch should not be stirred unless necessary since the swollen granules are easily broken. The broken grains and fragments will thin out the mixture. As the cooked starch mixture cools there is a marked increase in stiffness of the gel formed. This is due to the decrease in kinetic energy which keeps the molecules from reassociating.

4.4.2 Proportion of Starch

More concentrated disappearance of starch show higher viscosity at lower temperature than do less concentrated mixtures because of the larger number of granules that can swell in the early stages of gelatinization. Each type of starch gelatinizes over a characteristic temperature range. although this range may be effected by starch concentration.

4.4.3 Agitation of Stirring

Stirring while cooking a starch mixture is desirable in the early stages for obtaining uniform consistency. However if agitation too intense or continue too long it accelerates the breakdown or rupturing of the starch and decreases viscosity and may give a pasty mouth feel.

4.4.4 Addition of other Ingredients

Addition of sugar decreases the thickness of the cooked product. Sugar limits the swelling of starch grains whiling competing with them for water. In addition, it elevates the temperature at which the starch grain begins to thicken a liquid. It also makes the swollen grains more resistant to mechanical rupture after they are gelatinized. In a recipe calling for a large amount of sugar, only a part of the sugar need be added before cooking. After the starch mixture has been cooked, the remainder of the sugar can be added with much less effect on viscosity.

Acid in the form of vinegar or lime juice reduces the thickness of hot starch paste and the firmness of cooled paste. The decrease in thickness and stiffness has been attributed in part to fragmentation of swollen granules and hydrolysis of starch molecules. Acid and heat catalyze the hydrolysis of starch to dextrins. Acid hydrolyzed starch, when it is boiled hydrates at lower temperature than the unmodified starch. Acid thinned starch is often used in confectionery industry.

In cooked starch mixtures like custards containing fruits some amount of thinning occurs. When a high concentration of sugar is also present in a starch paste, the sugar may help to decrease the effect of acid because sugar limits the swelling of starch granules and starch molecules are therefore not as available for hydrolysis of acid. Acid juices such as lemon juice can also be added after the starchy paste has been cooked. This limits the acid's contact with starch molecules.

The presence of fats and proteins which tend to coat starch granules and thereby delay hydration also lowers the rate of viscosity development.

4.5 Degree of Retrogradation of Starch

Starch retrogradation has been used to describe changes in physical behavior following gelatinization. It is the process that occurs when starch molecules reassociate and form an ordered structure such as double helices during storage. In an initial step, two chains may associate. Ultimately, under favorable conditions, a crystalline order appears and physical phase separation occurs. Retrogradation is important in industrial use of starch, as it can be a desired end point in certain applications but it also causes instability in starch pastes. Structural modification, either by genetic means to change the pathway of starch biosynthesis or by means of chemical or physical modification of starch, has been employed to alter the process of retrogradation.

4.5.1 Factors Effecting Retrogradation

Starch retrogradation is influenced by the botanical source (e.g., cereal starch vs. tuber starch) and the fine structure of amylopectin (e.g., chain length and distribution). The amylose: amylopectin ratio affects the kinetics of retrogradation. In nonmutant genotype starches, the amylose is responsible for short term (<1 day) changes.The amylopectin molecule is responsible for longer term rheological and structural changes of starch gels. Water content in the starch gel and storage temperature can affect the rate and extent of starch retrogradation.

4.6 Summary

Starch is the reserve carbohydrate of plants ad occurs as granules in the cell in plastids, separated from the cytoplasm. Among food carbohydrates, starch occupies a unique position. It is the major carbohydrate storage material in many higher plants and is considered the second largest natural biopolymer next to cellulose. Starch is deposited in plant organs in the form of granules that are relatively dense, insoluble in cold water, and range from 1 to 100 ì m in size depending on the plant species. Some fresh plant crops, such as corn, potato, and sweet potato, contain about 15% or more starch. Nuts such as chestnuts contain up to 33% starch. Starch also exists in the stem-pith (e.g., sago) and fruits (e.g., banana). The starch content increases with the degree of refinement in milled products. Most starches are composed of a mixture of two molecular entities (polysaccharides), a linear fraction, amylose, and a highly branched fraction, amylopectin.

Starch granules do not dissolve readily in cold water but they will form a temporary suspension with the starch tending to settle out as soon as the mixture is allowed to stand. When heated with water the granules. As the temperature increases of the starch water mixture, the hydrogen bonding decreases for both the starch-starch bonds and water-water bonds and the size of the particles diminishes. Acid in the form of vinegar or lime juice reduces the thickness of hot starch paste and the firmness of cooled paste. Starch retrogradation has been used to describe changes in physical behavior following gelatinization. It is the process that occurs when starch molecules reassociate and form an ordered structure such as double helices during storage.

4.7. Self Assessment Questions

1 What are the various types of starches?
2 Explain about the starch chemistry
3 Structure of amylase and amylopectin
4 What are the different factors effecting properties of starch?
5 What is gelatinization of starch?
6 Explain about degree of retrogradation of starch?

4.8 Reference Books:

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2 John M. deMan, Principles of food chemistry, Third edition, 1999
3 Upadhyay and Upadhyay, Biophysical chemistry, 2006
4 Albert L. Lehninger and David L.Nelson Principles of Biochemistry, 5th edition
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7 Srilakshmi. B. Food Science

Dr.P. KIRANMAYI

Lesson - 5

PROTEIN CHEMISTRY

5.0 Objective

After going through this lesson you will learn:

- The properties of proteins
- Structure of proteins
- Factors effecting gluten formation

Structure

5.1 Introduction

Proteins are macromolecules found in all biological systems, from lower prokaryotes to higher eukaryotes. They occupy a prominent position in living cells, both quantitatively and qualitatively, which accounts for the origin of their name derived from the Greek word proˆtos, meaning 'first rank of importance'. Quantitatively, proteins are the most abundant class of biomolecules since they represent over 50% of the dry weight of cells, far more than other important biopolymers such as nucleic acids, polysaccharides or lipid assemblies. Each organism contains a large variety of specific proteins, according to the number of the corresponding genes present in chromosomes. This number varies from a few hundreds in certain bacterial species to several thousands in animals and man. Qualitatively, proteins are involved in virtually all biological processes. Thus, most chemical reactions occurring in life forms are catalyzed by specific proteins called enzymes that are able to increase reaction rates by several orders of magnitude.

Proteins can also transport and store a wide array of ions and small molecules as well as electrons. They possess hormonal activity and, in the form of antibodies which distinguish between self and nonself, they defend organisms against intruders. All proteins are polymers composed of the same building blocks, the amino acids, which are covalently joined together by amide links, known as peptide bonds. They differ only in the number, the nature, and the sequential order of their constituent amino acids. To understand the functional diversity of proteins, it is important, first, to appreciate the physicochemical properties of the different amino acids, even though the properties of a protein molecule are hugely more complex than the sum of the properties of its different constituent amino acids.

5.2 Nature and types of proteins

Plant proteins occur in cells conjugated with lipid, carbohydrate, and other molecules, but only those conjugated proteins which are not readily dissociated are isolated intact. Most proteins are readily denatured by a wide variety of reagents and conditions, and the utmost care must be used to avoid reaction.

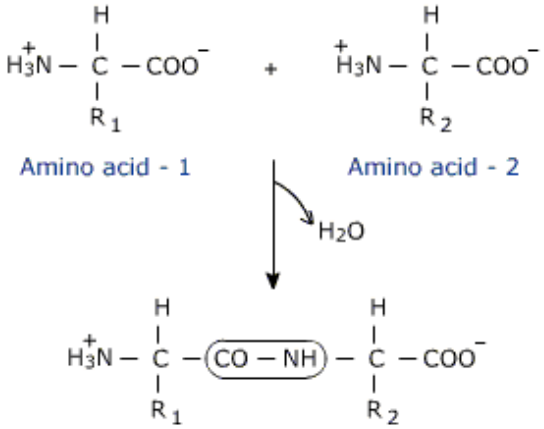
Milk is one of the excellent sources of protein in man's diet. Various proteins that are present in milk are: Casein is present in cow's milk to the extent of 3.0 to 3.5 percent, in human milk 0.3 to 0.6 percent. Globulin or lactoglobulin and albumin or lactalbumin is whey proteins. Colostrum is the first secretion of the mammary gland on parturition and differs markedly from milk in its protein composition. During the first few days after the birth of the calf, the composition of the lacteal secretion gradually changes until that characteristic of milk appears. On birth of the calf the colostrums contains approximately 17.5 percent protein with about 5 percent of this casein. The most striking difference is in the globulin fraction that carries the antibodies which give protection against certain diseases. Egg proteins are ovalbumin, conalbumin, ovomucoid, globulins G_2 and G_3, ovomucin, avidin, lipoviellin and lipovitellenin.

Lean meat consists essentially of muscle fibers, connective tissues, and fat cells. The fibers of ordinary meat are striated and have a sheath, known as the Sarcolemma, made up chiefly of a protein related to elastin, insoluble in ordinary neutral reagents and belonging therefore to the albuminoids. Within the sarcolemma is contained the meat juice containing several proteins, of which Myosin is the most important. Elastin and Collagen are the chief constituents of cormective tissue.

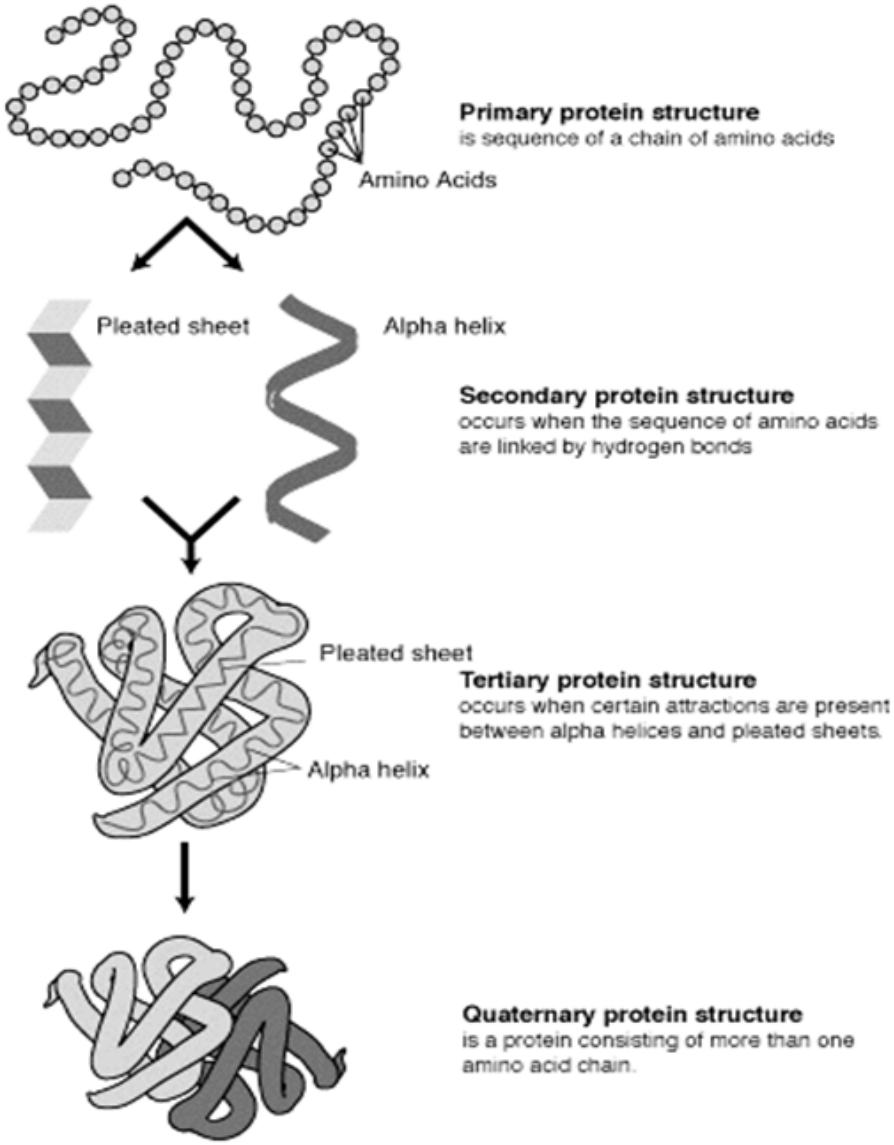
Flesh is any edible part of the striated muscle of an animal. The term animal as herein used, indicates a mammal, a fowl, a fish, a crustacean, a mollusk, or any other animal used as a source of food. The protein-N content of fish muscle tissue is between 2–3%. The amino acid composition, when compared to that of beef or milk casein, reveals the high nutritional value of fish proteins. The sarcoplasma protein accounts for 20–30% of the muscle tissue total protein. The contractile apparatus accounts for 65–75% protein; the connective tissue of teleosts is 3%; and of elasmobranchs, such as sharks and rays (skate or rocker), is up to 10%. The individual protein groups and their functions in muscle tissue of mammals also apply to fish.

5.3 Structure of Proteins

The potential configuration of protein molecules is so complex that many types of protein molecules can be constructed and are found in biological materials with different physical characteristics. Globular proteins are found in blood and tissue fluids in amorphous globular form with very thin or non-existent membranes. Collagenous proteins are found in connective tissue such as skin or cell membranes. Fibrous proteins are found in hair, muscle and connective tissue. Crystalline proteins are exemplified by the lens of the eye and similar tissues. Enzymes are proteins with specific chemical functions and mediate most of the physiological processes of life. Several small polypeptides act as hormones in tissue systems controlling different chemical or physiological processes. Muscle protein is made of several forms of polypeptides that allow muscular contraction and relaxation for physical movement.



Peptide bond



Structure of proteins

5.4 Properties of Proteins

Proteins can also be characterized by their chemical reactions. Most proteins are soluble in water, in alcohol, in dilute base or in various concentrations of salt solutions. Proteins have the

characteristic coiled structure which is determined by the sequence of amino acids in the primary polypeptide chain and the stereo configuration of the radical groups attached to the alpha carbon of each amino acid. Proteins are heat labile exhibiting various degrees of liability depending upon type of protein, solution and temperature profile. Proteins can be reversible or irreversible, denatured by heating, by salt concentration, by freezing, by ultrasonic stress or by aging. Proteins undergo characteristic bonding with other proteins in the so-called plastein reaction and will combine with free aldehyde and hydroxyl groups of carbohydrates to form Maillard type compounds.

5.5 Amino acid Composition

Amino acids joined together by peptide bonds form the primary structure of proteins. 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 behavior during processing. Of the 20 amino acids, only about half are essential for human nutrition. The amounts of these essential amino acids present in a protein and their availability determine the nutritional quality of the protein. In general, animal proteins are of higher quality than plant proteins. Egg protein is one of the best quality proteins and is considered to have a biological value of 100. It is widely used as a standard, and protein efficiency ratio (PER) values sometimes use egg white as a standard. Cereal proteins are generally deficient in lysine and threonine. Soybean is a good source of lysine but is deficient in methionine. Cottonseed protein is deficient in lysine and peanut protein in methionine and lysine.

Product	Protein (g/100g)
Meat: beef	16.5
Pork	10.2
Chicken (light meat)	23.4
Fish: haddock	18.3
Cod	17.6
Milk	3.6
Egg	12.9
Wheat	13.3
Bread	8.7
Soy beans: dry, raw	34.1
Cooked	11.0
Rice: white, raw Cooked	6.7
Potato	2.0
Corn	10.0

Protein content of some selected foods

5.6 Chemical Changes

During processing and storage of foods, a number of chemical changes involving proteins may occur some of these may be desirable, others undesirable. Such chemical changes may lead to compounds that are not hydrolyzable by intestinal enzymes or to modifications of the peptide side chains that render certain amino acids unavailable. Mild heat treatments in the presence of water can significantly improve the protein's nutritional value in some cases. Sulfur-containing amino acids may become more available and certain antinutritional factors such as the trypsin inhibitors of soybeans may be deactivated. Excessive heat in the absence of water can be detrimental to protein quality; for example, in fish proteins, tryptophan, arginine, methionine, and lysine may be damaged.

A number of chemical reactions may take place during heat treatment including decomposition, dehydration of serine and threonine, loss of sulfur from cysteine, oxidation of cysteine and methionine, cyclization of glutamic and aspartic acids and threonine. The nonenzymic browning, or Maillard, reaction causes the decomposition of certain amino acids. For this reaction, the presence of a reducing sugar is required. Heat damage may also occur in the absence of sugars. The Maillard reaction leads to the formation of brown pigments, or melanoidins, which are not well defined and may result in numerous flavor and odor compounds. The browning reaction may also result in the blocking of lysine. Lysine becomes unavailable when it is involved in the Amadori reaction, the first stage of browning reacting with free radicals formed by lipid oxidation. Methionine can react with a lipid peroxide to yield methionine sulfoxide.

5.7 Proteins of Wheat

5.7.1 Gluten

It can be readily prepared by adding 60 to 65 percent water to a hard wheat flour, allowing the dough to stand approximately 30 minutes, and then washing out the starch granules and soluble compounds under a stream of water. A tough, elastic, gummy product is obtained which consists of approximately two-thirds water and one third protein. There are also small amounts of lipids, starch and ash.

5.8 Factors Effecting Gluten Formation

5.8.1 Mechanical Action

The mechanical action to which the dough is subjected either through mixing or kneading is important for the development of the gluten. Elastic strands of rubbery gluten form as the flour is mixed with water. Under mixing produces dough in which there has not been sufficient gluten development to retain the gas well and the result is loaf which has poor volume and is heavy. Over mixing or rough handling of the dough before panning has been reported to decrease the volume of the loaf. When flour and water are mixed, an increase in plasticity occurs as the gluten is developed. But if mixing is continued, a decline in plasticity occurs and the dough finally becomes slack and sticky.

5.8.2 Proteolytic Enzymes

These are a group of enzymes present in wheat and flour which catalyze the hydrolysis of proteins. They are also present in malted wheat flour, malted barley, malt extract and yeast. If too

much of these proteolytic enzymes are present, too much hydrolysis occurs and the dough becomes sticky, difficult to machine in the mixers, and yield bread of poor volume.

5.8.3 Oxidation

If an oxidizing agent is added either to flour or to gluten, the strength of the gluten is increased. If relatively large amounts are added, the gluten becomes tough with little elasticity. Reducing agents have the opposite effect. They cause a decrease in the strength of the gluten, making it more extensible and sticky.

5.9 Dough

When flour is mixed with water, a change takes place in the physical-chemical properties of the gluten. It has been customary to consider gluten the product of two protein fractions of wheat-gliadin and glutenin. It is in wheat flour and only in wheat flour that this elastic, rubbery protein develops when it is stirred with water. The ability of dough to retain gas depends directly on the amount and quality of th gluten developed. The volume or lightness of a wheat loaf is therefore directly dependent on the factors which contribute to the development of gluten and which influence its elasticity. Dough made of other cereals is not able to retain all of the gas generated and consequently are heavy and coarse textured.

5.10 Summary

Proteins are involved in virtually all biological processes. Thus, most chemical reactions occurring in life forms are catalysed by specific proteins called enzymes that are able to increase reaction rates by several orders of magnitude. During processing and storage of foods, a number of chemical changes involving proteins may occur some of these may be desirable, others undesirable. A number of chemical reactions may take place during heat treatment including decomposition, dehydration of serine and threonine, loss of sulfur from cysteine, oxidation of cysteine and methionine, cyclization of glutamic and aspartic acids and threonine.

The mechanical action to which the dough is subjected either through mixing or kneading is important for the development of the gluten. Elastic strands of rubbery gluten form as the flour is mixed with water. Under mixing produces dough in which there has not been sufficient gluten development to retain the gas well and the result is loaf which has poor volume and is heavy. If an oxidizing agent is added either to flour or to gluten, the strength of the gluten is increased. If relatively large amounts are added, the gluten becomes tough with little elasticity. When flour is mixed with water, a change takes place in the physical-chemical properties of the gluten. It has been customary to consider gluten the product of two protein fractions of wheat-gliadin and glutenin.

5.11 Self Assessment Questions

1 What is the nature of a protein?
2 What are the properties of proteins?
3 Explain in detail about the chemical changes of proteins?
4 What are the wheat proteins?
5 Explain about various factors that effect the gluten formation?
6 What is a dough?

5.12 Reference Books:

1 H.D.Belitz, W.Grosch and P.Schieber. Food Chemistry 4th revised and extended edition 2009
2 John M. deMan, Principles of food chemistry, Third edition, 1999
3 Upadhyay and Upadhyay, Biophysical chemistry, 2006
4 Albert L. Lehninger and David L.Nelson Principles of Biochemistry, 5th edition
5 Lillian Hoagland Meyer, Food chemistr, Litton Educational Publishing inc, U.S.A.

Dr.P.KIRANMAYI

Lesson - 6

LIPID CHEMISTRY - I

6.0 Objective

After going through this lesson you will learn:

- The composition of lipids
- Physical and chemical properties of lipids

Structure

6.1 Introduction

It has been difficult to provide a definition for the class of substances called lipids. Early definitions were mainly based on whether the substance is soluble in organic solvents like ether, benzene, or chloroform and is not soluble in water. For example, monoglycerides of the short chain fatty acids are undoubtedly lipids, but they would not fit the definition on the basis of solubility because they are more soluble in water than in organic solvents. Instead of trying to find a definition

that would include all lipids, it is better to provide a scheme describing the lipids and their components. These are mostly esters of fatty acids and glycerol. Up to 99 percent of the lipids in plant and animal material consist of such esters, known as fats and oils. Fats are solid at room temperature, and oils are liquid.

6.2 Composition and Classification

The composition of a fatty acid can be described by two numbers separated by a colon. The first number indicates the number of carbon atoms in the fatty acid chain, the second number indicates the number of double bonds. Thus, 4:0 is short for butyric acid, 16:0 for palmitic acid, 18:1 for oleic acid, etc. The two numbers provide a complete description of a saturated fatty acid. For unsaturated fatty acids, information about the location of double bonds and their stereo isomers can be given as follows: oleic acid (the *cis* isomer) is 18:lc9; elaidic acid (the *trans* isomer) is I8:lt9.

The numbering of carbon atoms in fatty acids starts normally with the carboxyl carbon as number one. In some cases polyunsaturated fatty acids are numbered starting at the methyl end; for instance, linoleic acid is represented as 18:2n-6 and linolenic acid 18:3n-3. These symbols indicate straight-chain, 18-carbon fatty acids with two and three methylene interrupted *cis* double bonds that start at the sixth and third carbon from the methyl end, respectively. These have also been described as 006 and co3. The reason for this type of description is that the members of each group *n-6* or *n-3* are related biosynthetically through processes involving desaturation, chain elongation, and chain shortening. Triglycerides can be abbreviated by using the first letters of the common names of the component fatty acids. SSS indicates tristearin, PPP tripalmitin, and SOS a triglyceride with two palmitic acid residues in the 1 and 3 positions and oleic acid in the 2 position.

In some cases, glyceride compositions are discussed in terms of saturated and unsaturated component fatty acids. In this case, S and U are used and glycerides would be indicated as SSS for trisaturated glyceride and SUS for a glyceride with an unsaturated fatty acid in the 2 position. In other cases, the total number of carbon atoms in a glyceride is important, and this can be shortened to glycerides with carbon numbers 54, 52, and so on. A glyceride with carbon number 54 could be made up of three fatty acids with 18 carbons, most likely to happen if the glyceride originated from one of the seed oils. A glyceride with carbon number 52 could have two component fatty acids with 18 carbons and one with 16 carbons. The carbon number does not give any information about saturation and unsaturation.

The general classification of lipids that follows is useful to differentiate lipids in foods.

Simple Lipids:

Ester of fatty acids with alcohol.

- Fats: Esters of fatty acids with glycerol – triacylglycerols
- Waxes: Esters of fatty acids with long-chain alcohols other than glycerols (e.g., myricyl palmitate, cetyl palmitate, vitamin A esters, and vitamin D esters)

Compound Lipids

Compounds containing groups in addition to an ester of a fatty acid with an alcohol.

• Phospholipids: Glycerol esters of fatty acids, phosphoric acids, and other groups containing nitrogen (e.g., phosphatidyl choline, phosphatidyl serine, phosphatidyl ethanolamine, and phosphatidyl inositol).

• Cerebrosides: Compounds containing fatty acids, a carbohydrate, and a nitrogen moiety (e.g., galactocerebroside and glucocerebroside)

• Sphingolipids: Compounds containing fatty acids, a nitrogen moiety, and phosphoryl group (e.g., sphingomyelins)

Derived Lipids

Derived lipids are substances derived from neutral lipids or compound lipids. They have the general properties of lipids – examples are fatty acids, long chain alcohols, sterols, fat-soluble vitamins, and hydrocarbons.

6.3 Importance of Analyses

Health concerns require the measurement of such parameters as cholesterol and phytosterol contents and amounts of *trans*, n-3/ù 3, saturated, mono- and polyunsaturated fatty acids. Lipid stability impacts not only the shelf life of food products, but also their safety, since some oxidation products (e.g., malondialdehyde, cholesterol oxides) have toxic properties. Another area of interest is the analysis of oils and fats used in deep fat frying operations. Total polar materials or acid value are used as quality standards in deep fat frying oil. Finally, the development of food ingredients composed of lipids that are not bioavailable (e.g., sucrose polyesters such as Olestra) or lipids not contributing the normal 9 Cal/g to the diet (e.g., short- and medium-chain triglycerides such as Salatrim and Caprenin) accentuates the need to characterize the lipids present in food.

6.4 Methods for Bulk Oils and Fats

Numerous methods exist to measure the characteristics of fats and oils. Some methods (e.g., titer test) have limited use for edible oils (in contrast to soaps and industrial oils). Other methods may require special apparatus not commonly available or may have been antiquated by modern instrumental procedures [e.g., volatile acid methods (Reichert–Meissl, Polenske, and Kirschner values) have been replaced largely by determination of fatty acid composition using gas chromatography).

6.5 Physical Properties

The physical properties of the natural fats and oils are often used to identify them. Usually more than one property is measured so that the identification can be made with some assurance since natural fats and oils vary somewhat in their properties. Their composition is not constant but varies slightly with climate, soil and variety of vegetable oils and with nutrition, season and breed for animal oils.

6.5.1 Melting Point

Fats do not melt sharply but soften over a range of temperatures, and is therefore impossible to apply the melting point technique, used in the identification of pure organic compounds, to them. If a fat, a fatty acid, or some esters of fatty acids are heated very slowly, they will melt, exist as a liquid as the temperature rises, and then solidify again. A second melting will then occur at a high

temperature. If the material is then chilled rapidly, it will melt at a lower temperature but it is warmed again. Polymorphism, the occurrence of more than one crystalline form, explains this phenomenon.

Polymorphism is found in many long chain carbon compounds. It is important to the understanding of the melting-point behavior of fats, fatty acids, and their esters. Furthermore, polymorphism plays a significant role in any operation where fats are solidified. As a fat is warmed, the number of crystals distributed through the liquid fat diminishes and the amount of liquid increases so that the fat softens. If the number of crystals exceeds a critical amount, the fat will be hard and brittle and will lose plasticity.

On the other hand, if the amount of liquid exceeds a critical level, the fat will flow. Natural fats are complex mixtures of glycerides each with its own characteristic melting point. As the temperature of the fat is raised, the melting point of first one and then another of these glycerides is exceeded. Eventually a temperature s reached at which all of the glycerides have melted and the fat is liquid. The temperature at which this occurs is not sharply defined.

The *softening point* of a fat is sometimes determined as a means of identification, but it cannot be applied to all fats. Capillary tubes are filled with oil and packed in ice over night so that the oil can solidify and come to equilibrium. The capillary tubes are clamped to a thermometer and submerged in a beaker of water. The temperature is slowly raised and the temperature at which the column of fat rises in the capillary tube is called the softening point.

The *slipping point* is anther empirical method used to identify some natural fats and fat compounds. Small brass cylinders, filled with the solid fat, are suspended in a bath close to the thermometer. As the bath is stirred, the temperature is slowly raised. The point at which the fat rises in the cylinder, or slips, is recorded as the slip point. The slip point is related to the composition of the fat. The shot melting point is the temperature at which a small lead shot will fall through a sample. This method has some usefulness.

The *specific gravity* of oils and fats is determined by the usual methods. The temperature is carefully controlled since significant changes in these compounds occur in short ranges of temperature. The specific gravity of a fat or oil is usually measure at 25^0c, but it may be necessary to use temperatures of 40^0c or even 60^0c for high melting fats. In general, either unsaturation of the fatty acid chains or increase in chain length of fatty acid residues tends to increase the specific gravity. The index of refraction is the degree of deflection of a beam of light that occurs when it passes from one transparent medium to another. The refractive indices of fats and oils are often measured both cause they can be rapidly and accurately determined and because they useful in identification of these substances and the testing of their purity. An Abbe Refractometer with temperature control is used and the measurement is usually at 25^0c. with high melting fats 40^0c or even 60^0c can be used, but temperature must be controlled and noted. The index of refraction decreases as the temperature rises; however it increases with increase in the length of the carbon chains and also with the number of double bonds present.

The *smoke point* is the temperature which a fat or oil gives off a thin bluish smoke. The flash point is the temperature at which the mixtures of vapour with air will ignite; the fire point is the temperature at which the substance will sustain continued combustion. The temperature vary with the amount of free fatty acids present in an oil or fat is important. The smoke point of a fat used for deep fat frying decreases with use of the fat. Fats and oils with low molecular weight fatty acids have low smoke, flash, and fire points. The number of double bonds present has little effect

on the temperature required. Smoke, flash and fire points are particularly useful in connection with fats used for any kind of frying.

6.5.2 Turbidity Point

The turbidity point of oil is determined by cooking a mixture of it has a solvent in which it has a limited solubility. The mixture is warmed until complete solution occurs and then slowly cooled until the oil begins to separate and turbidity occurs. The temperature at which turbidity first is detectable is known as the turbidity point.

6.5.3 Refractive Index

The refractive index (RI) of an oil is defined as the ratio of the speed of light in air (technically, a vaccum) to the speed of light in the oil. When a ray of light shines obliquely on an interface separating two materials, such as air and oil, the light ray is refracted in a manner defined by Snell's law, as shown in equation

$$\grave{e}1n1 = \grave{e}2n2$$

where:

$\grave{e}1$ = angle of the incident light

$n1$ = refractive index of material 1

$\grave{e}2$ = angle of the refracted light

$n2$ = refractive index of material 2

If the angles of incidence and refraction and the refractive index (n) of one of the two materials are known, the refractive index of the other material can be determined. In practice, the $\grave{e}1$ and $n1$ are constant, so $n2$ is determined by measuring $\grave{e}2$.

Because the frequency of light affects its refraction (violet light is refracted more than red light), white light can be dispersed or split after refraction through two materials of different refractive indexes (explaining the color separation of diamonds and rainbows). Refractometers often use monochromatic light (or nearly monochromatic light from the sodium doublet D line, that has 589.0 and 589.6 nm wavelengths or light emitting diodes to provide 589.3 nm) to avoid errors from variable refraction of the different wavelengths of visible light.

6.6 Chemical Properties

A number of chemical tests have been evolved during the years of study of oils and fats which are based on the partial determination of the chemical composition of the oil or fat. These tests serve both to identify the fat and to detect the presence of adulteration. All oils and fats show some range of values; therefore sometimes more than one test is necessary. A few of the most commonly used test are given below.

6.6.1 The Reichert Meissl Number

It is defined as the number of milliliters of 0.1N alkali (such as potassium hydroxide) required to neutralize the volatile water-soluble fatty acids in a 5 g sample of fat. The volatile acids will be those in the range of molecular weights from butyric (C_4) to myristic (C_{14}) acid. The Reichert

Meissl test determines the amount of butyric acid and caproic acids which are readily soluble in water and the caprylic and capric acids which are slightly soluble. The Reichert Meissl Number is particularly valuable in detecting adulteration in butter. Although this varies for butter with season, nutrition, and time in the lactional cycle of the cow it is usually between 24 and 34, higher than other edible oils.

6.6.2 Polenske Number

It is the number of milliliters o 0.1 *N* alkali necessary to neutralize the volatile, water-insoluble fatty acids which are present in a 5 g sample. These two determinations are readily run on the same sample of fat.

6.6.3 The Saponification Number

It is defined as the number of milligrams of potassium hydroxide required to saponify 1 g of fat or oil. When potassium hydroxide reacts with a triglyceride, three moles of potassium hydroxide react with one mole of fat. If triglyceride contains low molecular weight fatty acids, the number of molecules present in a 1 g sample of the fat will be greater than if the fatty acids have long carbon chains and high molecular weight. The fat with the low molecular weight fatty acids will consequently have a high saponification number. We find that butter with its unusually high percentage of butyric acid has the highest saponification.

6.6.4 The Iodine Number

It is the number of grams of iodine or iodine compound absorbed 100 g of fat. The double bonds present in the unsaturated fatty acids react readily with iodine or certain iodine compounds to form an addition compound even while the fatty acid is combined with glycerol in the fat. The Iodine Number is therefore a measure of the extent of unsaturation of the fatty acids present in a fat. While oleic acid contains one double bond in its 18 carbon chain, linolenic acid contains three double bonds in its 18 carbon chain. Thus a molecule of fat containing one oleic acid can absorb or react with only one third as much iodine as a molecule of fat containing on linolenic acid residue.

The Iodine Number is determined by dissolving a weighed sample of fat in chloroform or carbon tetrachloride and adding an excess of halogen. After standing in the dark for a controlled period of time the excess, un reacted iodine is measured by thiosulfate titration.

6.6.4.1 Applications

Iodine value is used to characterize oils, to follow the hydrogenation process in refining, and as an indication of lipid oxidation, since there is a decline in unsaturation during oxidation. The calculated value tends to be low for materials with a low iodine value and for oils with greater than 0.5% unsaponifiable material (e.g., fish oils). The Wijs iodine procedure uses ICl and the Hanus procedure uses IBr. The Wijs procedure may be preferable for highly unsaturated oils as it reacts faster with the double bonds.

6.6.5 The Acetyl Value

The acetyl value of fats or oils defined as the amount of KOH in mg required for the neutralization of the acetic acid obtained by the saponification of 1 g of the acetylated product.

The process consists in acetylating the oil or fat with a measured quantity of acetic anhydride in pyridine decomposing the excess anhydride by boiling with water and then, after the addition of sufficient butyl alcohol to give a homogeneous solution, titrating with alcoholic alkali. A control test with the acetic anhydride and pyridine without the oil or fat provides a measure of the acetic anhydride available for acetylation; a similar test with the oil or fat and the pyridine without the acetic anhydride provides a measure of the free fatty acid present. From the figures obtained, the acetyl value or the hydroxyl value of the fat is calculated.

6.6.6 Kirschner Value

It measures the potential amount of soluble silver salts in the Reichert Meissl distillate. Silver butyrate is soluble in water while the silver salts of the other volatile, water-soluble fatty acids are relatively insoluble. The neutralized distillate from the Reichert Meissl determination is treated with silver sulfate and filtered. The filtrate is acidified with sulfuric acid and distilled. The distillate is carefully collected and titrated with 0.1 N alkali, either sodium, potassium, or barium hydroxide.

6.7 Summary

The physical properties of the natural fats and oils are often used to identify them. These are mostly esters of fatty acids and glycerol. Up to 99 percent of the lipids in plant and animal material consist of such esters, known as fats and oils. Fats are solid at room temperature, and oils are liquid. The composition of a fatty acid can be described by two numbers separated by a colon. The first number indicates the number of carbon atoms in the fatty acid chain, the second number indicates the number of double bonds. The physical properties of the natural fats and oils are often used to identify them. Usually more than one property is measured so that the identification can be made with some assurance since natural fats and oils vary somewhat in their properties. Their composition is not constant but varies slightly with climate, soil and variety of vegetable oils and with nutrition, season and breed for animal oils.

The specific gravity of oils and fats is determined by the usual methods. The temperature is carefully controlled since significant changes in these compounds occur in short ranges of temperature. The specific gravity of a fat or oil is usually measure at 25^0c, but it may be necessary to use temperatures of 40^0c or even 60^0c for high melting fats. A number of chemical tests have been evolved during the years of study of oils and fats which are based on the partial determination of the chemical composition of the oil or fat.

6.8 Self Assessment Questions

1 What is the composition of lipids?
2 What are the physical properties of lipids?
3 What are the chemical properties of lipids?
4 Define melting point and turbidity point of lipids?
5 Write short notes on the following
   a. Kirschner value
   b. Iodine number
   c. Saponification value
   d. Reichert Meissl number

6.9 Reference Books:

1 H.D.Belitz, W.Grosch and P.Schieber. Food Chemistry 4th revised and extended edition 2009
2 John M. DeMan, Principles of food chemistry, Third edition, 1999
3 Upadhyay and Upadhyay, Biophysical chemistry, 2006
4 Albert L. Lehninger and David L.Nelson Principles of Biochemistry, 5th edition
5 Lillian Hoagland Meyer, Food chemistry, Litton Educational Publishing inc, U.S.A.

Dr.P.KIRANMAYI

Lesson - 7

LIPID CHEMISTRY-II

7.0 Objective

After going through this lesson you will learn:

- The concept of rancidity
- Changes occur in oils during heating
- Hydrogenation of fats
- Shortening power of fats

Structure

7.1 Introduction

When fats and oils are stored they undergo flavour changes which markedly influence their market value. The flavour of rancidity is well known to everyone; and the problem of rancidity, although by no means completely not understood. Some oils and fats develop off-flavours before the onset of rancidity. This change is called reversion, and it, too, is important because it makes the fat or oil undesirable for food products.

7.2 Rancidity

It has been known for many years that fats and oils slowly take up oxygen for a period of time before it is possible to detect the flavour of the products of rancidity. This period is called the induction period and it is followed by a second period in which the uptake is much more rapid. Rapid oxidation often continues for an extended period of time after which the rate fall off. The length of each period is markedly affected by many factors for each fat. And the course of the oxidation can apparently take a number of paths.

Temperature, moisture, the amount of air in contact with fat, light particularly that in the ultraviolet or near ultraviolet, as well as the presence or absence of antioxidants and prooxidants influence the reaction. The uptake of oxygen and the onset of rancidity seem to be related to the unsaturation of the fat.

Vegetable fats, particularly those from seeds, show a marked resistance to the onset of rancidity. Some seeds, if they are not bruised or crushed, can be stored for years without any change in the fats. But in general animal fats deteriorate rapidly.

The uptake of oxygen and the onset of rancidity seems to be related to the unsaturation of the fat, although this has been exceedingly difficult to show by direct comparison of natural fats. The oxidation is not however, a simple oxidation of the double bond. Many attempts to analyze the volatile compounds which can be smelled in a rancid fat show that a very complex mixture of compounds is formed. The products formed during the induction period have for many years been called peroxides.

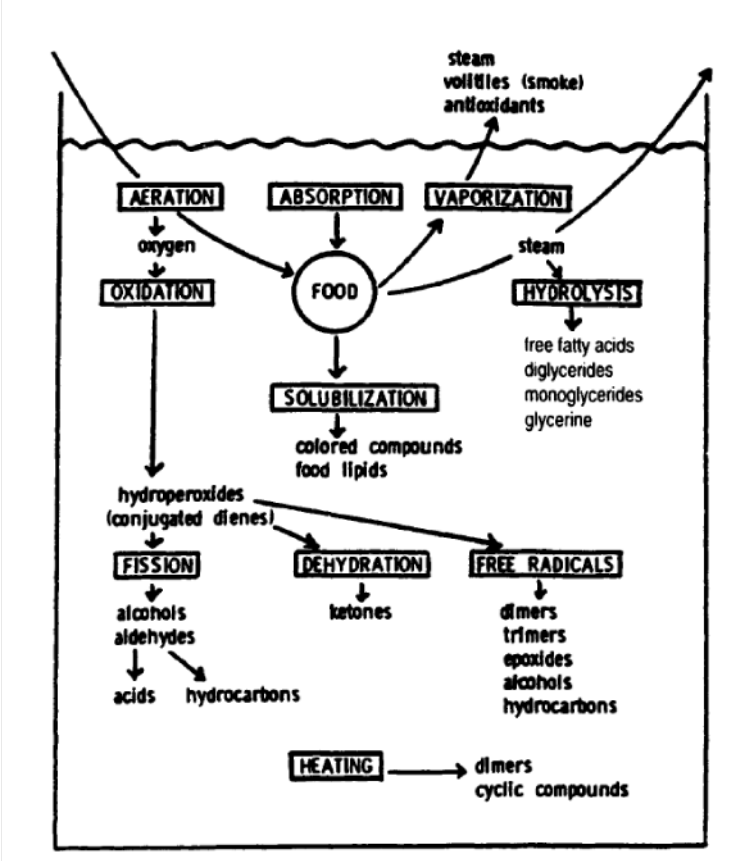
7.3 Heating

Fats and oils are heated during commercial processing and during frying. Heating during processing mainly involves hydrogenation, physical refining, and deodorization. Temperature used in these processes may range from 12O ^{0}C to 27O ^{0}C. The oil is not in contact with air, which eliminates the possibility of oxidation. At the high temperatures used in physical refining and deodorization, several chemical changes may take place. These include randomization of the glyceride structure, dimer formation, *cis-trans* isomerization, and formation of conjugated fatty acids (positional isomerization) of polyunsaturated fatty acids.

Deep frying, where the food is heated by immersion in hot oil, is practiced in commercial frying as well as in food service operations. The temperatures used are in the range of 16O ^{0}C to 195 ^{0}C. At lower temperatures frying takes longer, and at higher temperatures deterioration of the oil is the limiting factor. Deep frying is a complex process involving both the oil and the food to be fried. Steam is given off during the frying, which removes volatile antioxidants, free fatty acids, and other volatiles.

Contact with the air leads to autoxidation and the formation of a large number of degradation products. The presence of steam results in hydrolysis, with the production of free fatty acids and partial glycerides. At lower frying temperatures the food has to be fried longer to reach the desirable color, and this result in higher oil uptake. Oil absorption by fried foods may range from 10 to 40 percent, depending on conditions of frying and the nature and size of the food.

Oils used in deep frying must be of high quality because of the harsh conditions during deep frying and to provide satisfactory shelf life in fried foods. The suitability of an oil for frying is directly related to its content of unsaturated fatty acids, especially linolenic acid. The higher the inherent stability, the less suitable the oil is for frying. The liquid seed oils, such as soybean and sunflower oil, are not suitable for deep frying and are usually partially hydrogenated for this purpose. Such hydrogenated oils can take the form of shortenings, which may be plastic solids or pourable suspensions.



Summary of chemical reactions occurring during deep frying

The stability of frying oils and fats is usually measured by an accelerated test known as the active oxygen method (AOM). In this test, air is bubbled through an oil sample maintained at 95 ^{0}C and the peroxide value is measured at intervals. At the end point the peroxide value shows a sharp increase, and this represents the AOM value in hours. Typical AOM values for liquid seed oils range from 10 to 30 hours; heavy-duty frying shortenings range from 200 to 300 hours.

Oil breakdown during frying can be caused by oxidation and thermal alteration. Oxidation can result in the formation of oxidized monomeric, dimeric, and oligomeric triglycerides as well as volatile compounds including aldehydes, ketones, alcohols, and hydrocarbons. In addition, oxidized sterols may be formed. Thermal degradation can result in cyclic monomeric triglycerides and nonpolar dimeric and oligomeric triglycerides.

Oil	Iodine value	Inherent stability
Soyabean	130	7.4
Sunflower	120	7.7
High-oleic sunflower	90	2.0
Corn	110	6.2
Cotton seed	98	5.2
Canola	110	5.4
Peamit	92	4.5
Lard	60	1.4
Olive	88	1.8
Palm	55	1.4
Palm olein	58	1.6
Palm stearin	35	1.0
Tallow	50	0.7
Palm kernel	17	0.5
coconut	9	0.4

Inherent stability of oil for use in frying

7.4 Hydrogenation

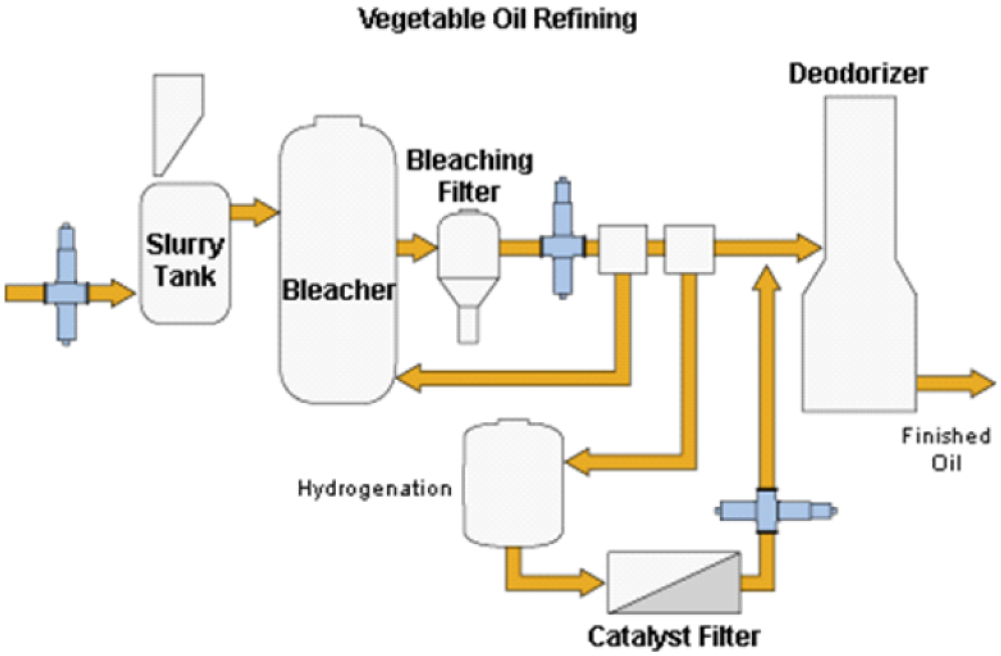
Hydrogenation of fats and oils is the process by which molecular hydrogen is added to double bonds in the unsaturated fatty acids of the glycerides. Economically, hydrogenation is a most important process since by its use the physical properties of a natural fat can be altered. Thus the physical properties of the products can be regulated so that many natural fats can be interchanged, so that liquid fats can be substituted for plastic fats, and an improvement occurs in the properties of natural plastic fats.

Hydrogenation occurs when hot oil saturated with hydrogen is brought in contact with an active catalyst, but the course of the reaction and its velocity is influenced by numerous factors. In most oils with a high content of C_{18} unsaturated acids the possible reactions are

- Linolenic to linoleic or isolinoleic
- Linoleic to oleic
- Oleic to stearic.

When the oil contains unsaturated acids with carbon chains of other lengths, hydrogen may added to these chains. The products may be the natural linoleic and oleic acids or they may

be the so called iso acids in which the double bond is in3a different position than that of the naturally occurring acid.



Hydrogenation of oils

When hot oil and catalyst are stirred together under an atmosphere of hydrogen, the properties of the final product are affected by temperature, rate of mixing, nature of the catalyst, concentration of the catalyst and pressure of hydrogen. A number of active metals are capable of catalyzing this reaction, but industrially nickel is used.

7.5 Interesterification

A recent development in the technology of lard may return this fat to its former position as a widely used and high priced fat. This is directed interesterification of lard which improves its plastic range and diminishes its tendency to graininess. Natural lard tends to develop fairly large crystals of disaturated glycerides that give a grainy texture when the lard is chilled and make it difficult to cream in batters and doughs.

Since the melting points of the disaturates are in the range of room temperature, the lord softens so much that it as little body and almost no plasticity at these temperatures. Lard has approximately 37 percent saturated fatty acids, principally stearic and palmitic. Under suitable conditions the glycerides present undergo interesterification with the rearrangement of the fatty acids in the glycerides. In directed interesterification the reaction is carried out at a temperature where all the glycerides exist in the liquid phase except the trisaturated esters that are solids. As

trisaturated glycerides are formed, they crystallize and are removed from the reaction mixture. The interesterification no longer is random, but is driven toward the formation of these solid trisaturates. The catalyst for the reaction in commercial interesterification is an alloy of potassium and sodium.

Lard is now interesterified commercially by a continuous process in which the catalyst is metred into the fat in very small particles. The temperature is carefully controlled and the fat is constantly agitated as crystallization occurs. The level of trisaturatedglycerides formed can be controlled by the temperature of the fat and the length of time allowed for crystallization. As the percentage of trisaturates increases, the resistance of the lard to softening at room temperature is increased or expressed in another way, the percentage of solids at a given temperature increases.

The catalyst is quenched by reaction with water and carbon dioxide. The amount of hydrolysis of fat is minimized by the introduction of carbon dioxide along with the water, which prevents the pH from rising too high. The soap formed is removed by centrifugation, washing and further centrifugation. The lard is then dried in a continuous vaccum drier.

The lard is usually hydrogenated to increase its resistance to rancidity and increase its consistency. It is deodorized and supplemented with antioxidants and monoglycerides before packaging.

Although the process is applied commercially to lard, interesterification of all natural fats will occur at elevated temperatures. Often the rate of reaction is slow and a considerable period of time is necessary to reach equilibrium. The change in the composition of the glycerides that is brought about by interesterification changes the physical properties of the fat. After interesterfication cocoa butter has a much higher melting point an da very different consistency. Some fats are more radically altered than others. Many catalysts have been used but the alkali metals and their alcoholates are most active.

7.6 Shortening Power of Fats

Shortening, fats and oils of animal or vegetable origin used in most doughs and batters to impart crisp and crumbly texture to baked products and to increase the plasticity, or workability, of doughs. Important commercial shortenings include butter, lard, vegetable oils, processed shortenings, and margarine. For most baking purposes, desirable characteristics include bland or pleasant flavour; freedom from objectionable odour; light or clear colour; a high degree of plasticity; long storage life; and good shortening power, or ability to weaken and lubricate the structure of baked products to produce tenderness. Firm fats produce flaky pastry; oils yield more compact pastry. The proportion of shortening in doughs and batters varies according to the product, with breads and rolls containing about 1–2 percent, cakes containing 10–20 percent, and piecrusts containing over 30 percent. Increasing shortening proportions incre0ases tenderness, but very high proportions may cause cakes to fall.

Butter, with yellow colour, solid consistency, and about 80 percent fat content, is valued for its sweet flavour, pleasant aroma, and ability to contribute great tenderness to baked products. It is popular for specialty breads, cookies, and pastries and is rolled into doughs from which flaky and tender pastries, such as Danish pastry and puff-paste products, are made. Because of its high cost, it is used, alone or in shortening mixtures, mainly in higher priced baked goods. It is fairly perishable, requiring storage at low temperature, and is not easily creamed (blended with sugar), producing cakes with lower volume and coarser grain than those made with more easily creamed shortenings.

Lard, obtained from the fatty tissue of hogs, has solid consistency, white colour, about 98 percent fat content, and mild, pleasing flavour and odour considered desirable in breads, crackers, cookies (sweet biscuits), and pie-crusts. Vegetable oils, obtained from such oil-bearing seeds as corn (maize), cottonseed, peanuts, palm nuts (coconuts), and soybeans, are 100 percent fat and remain liquid at fairly low temperatures. They are processed to achieve neutral to yellow colour and to eliminate odour or produce mild odour.

Oils are used mainly in rolls, breads, and other fairly hard baked goods and in chiffon and other cakes in which their liquid consistency is useful. Margarines are emulsions containing about 80 percent fat, from either animal or vegetable sources, plus water, salt, emulsifiers, and sometimes milk solids. They are white to yellow in colour, with neutral or butter like flavour and solid consistency. Margarine has a high melting point, produces tender products, and is especially popular for use in puff pastes.

7.7 Summary

Lipids are generally defined by their solubility characteristics rather than by some common structural feature. Lipids in foods can be classified as simple, compound, or derived lipids. The lipid content of foods varies widely, but quantitation is important because of regulatory requirements, nutritive value, and functional properties. When fats and oils are stored they undergo flavor changes which markedly influence their market value. Fats and oils are heated during commercial processing and during frying. Heating during processing mainly involves hydrogenation, physical refining, and deodorization. Temperature used in these processes may range from 120 ^{0}C to 270 ^{0}C. The oil is not in contact with air, which eliminates the possibility of oxidation. At the high temperatures used in physical refining and deodorization, several chemical changes may take place.

Hydrogenation of fats and oils is the process by which molecular hydrogen is added to double bonds in the unsaturated fatty acids of the glycerides. Economically, hydrogenation is a most important process since by its use the physical properties of a natural fat can be altered. A recent development in the technology of lard may return this fat to its former position as a widely used and high priced fat. This is directed interesterification of lard which improves its plastic range and diminishes its tendency to graininess. Shortening, fats and oils of animal or vegetable origin used in most doughs and batters to impart crisp and crumbly texture to baked products and to increase the plasticity, or workability, of doughs. Important commercial shortenings include butter, lard, vegetable oils, processed shortenings, and margarine. For most baking purposes, desirable characteristics include bland or pleasant flavour.

7.8 Self Assessment Questions

1 What is the composition of lipids?
2 What are the physical properties of lipids?
3 What are the chemical properties of lipids?
4 Define melting point and turbidity point of lipids?
5 What is iodine value and acetyl value of lipids?
6 Explain in detail about interesterification of fats?
7 What is shortening power of fats?

7.9 Reference Books:

1 H.D.Belitz, W.Grosch and P.Schieber. Food Chemistry 4th revised and extended edition 2009
2 John M. deMan, Principles of food chemistry, Third edition, 1999
3 Upadhyay and Upadhyay, Biophysical chemistry, 2006
4 Albert L. Lehninger and David L.Nelson Principles of Biochemistry, 5th edition
5 Lillian Hoagland Meyer, Food chemistry, Litton Educational Publishing inc, U.S.A.

Lesson - 8

VEGETABLES AND FRUITS

8.0 Objective

After going through this lesson you will learn:

- Composition and chemistry of vegetables and fruits
- Changes in post harvesting stages
- Various browning reactions and preventive methods

Structure

8.1 Introduction

Fruits and vegetables add enormously to the interest ad variety of our diet by their great range of colour and texture and by their complex aromas, giving each variety and even each individual a slightly different flavour. Nutritionally, fruits and vegetables are important because they contain large change in the aroma and even in the relative sourness and sweetness. So through the use of fresh and cooked fruits and vegetables, the possible variations in menu pattern are immense.

8.2 Vegetables

- Leaf vegetable (lettuce, mustard greens, chard, spinach, cabbage) are high in water and cellulose and low in calories and protein. They add valuable amounts of minerals and vitamins to the diet although they do not contain large amounts of most of these

nutrients. They are usually rich in iron and provitamin A, and often in the B complex. The raw leaves often contain appreciable amounts of ascorbic acid.

- Bulbs, roots, and tubers (potatoes, beets, turnips, carrots) are relatively high in water, moderate in cellulose, and contain an appreciable amount of available carbohydrate. The available carbohydrates are starches, glucose, and some sucrose. The amounts of the vitamins and minerals are not high but are valuable adjuncts to the diet.

- Flowers, buds and stems (cauliflower, asparagus celery) are relatively high in water and cellulose, but low in protein. They have moderate amounts of calcium and some are moderately rich in provitamin A. They all contribute small amounts of vitamins and minerals. A few have moderate amounts of ascorbic acid and riboflavin.

- Vegetable fruits (cucumbers, peppers, melons, tomatoes, pumpkin, egg plant) are relatively high in water and cellulose but low in calories and protein. Many contain valuable amounts of vitamins and small amounts of minerals. Some, such as the tomato, contain notable amount of ascorbic acid; others, such as green peppers and squash, for provitamin A and some, such as the tomato, for thiamine.

- Seeds (legumes, corn, rice) are relatively low in water and cellulose, containing a fair amount of protein and a large amount of starch. They are notable sources of the B complex vitamins and iron.

8.3 Composition of Fruits and Vegetables

All, with the exception of nuts and dates, are high in water with a range from approximately 70 percent for pears, bananas, figs, etc. to 98 percent of vegetable marrow. All, with the exception of legumes and legumes and nuts, are relatively low in protein. Although the quality of protein in vegetables and fruits is sometimes neglected, no cellular material ever exists without a certain amount. Protein varies from approximately 0.3 percent in apples to 4.4 percent in brussels sprouts.

All vegetables and fruits contain some carbohydrate. Part of the carbohydrate in fresh fruits is preset as cellulose and pectic substances in the cell walls, but these compounds are indigestible and not available to human body. Starch is present in almost all fruit and vegetables although it may disappear on ripening. Glucose, fructose, and sucrose are widely distributed, and sweet taste is dependent on their occurrence. Glucose, fructose, sucrose, and starches constitute the ‘available carbohydrate’ of fruits and vegetables, and the caloric value of the food depends in large measure on the concentration of these components.

8.4 Chemistry of Vegetables and Fruits

The composition of vegetables can vary significantly depending upon the cultivar and origin. The amount of dry matter in most vegetables is between 10 and 20%. The nitrogen content is in the range of 1–5%, carbohydrates 3–20%, lipids 0.1–0.3%, crude fiber about 1%, and minerals close to 1%. Some tuber and seed vegetables have a high starch content and therefore a high dry matter content. Vitamins, minerals, flavor substances and dietary fibers are important secondary constituents.

Fruit composition can be strongly influenced by the variety and ripeness. The major constituents are sugars, polysaccharides and organic acids, while N-compounds and lipids are

present in lesser amounts. Minor constituents include pigments and aroma substances of importance to organoleptic quality, and vitamins and minerals of nutritional importance. Nuts are highly variable in composition. Their moisture content is below 10%, N-compounds are about 20% and lipids are as high as 50%.

8.4.1 Polysaccharides

All fruits contain cellulose, hemicellulose (pentosans) and pectins. The building blocks of these polysaccharides are glucose, galactose, mannose, arabinose, xylose, rhamnose, fucose and galacturonic and glucuronic acids. The pectin fractions of fruits are particularly affected by ripening. A decrease in insoluble pectin is accompanied by an increase in the soluble pectin fraction. The total pectin content can also decrease. Starch is present primarily in unripe fruits and its content decreases to a negligible level as ripening proceeds. Exceptions are bananas, in which the starch content can be 3% or more even in ripe bananas, and various nuts such as cashew and Brazil nuts.

8.4.2 Amines

A number of aliphatic and aromatic amines are found in various fruits and vegetables They are formed in part by amino acid decarboxylation such as in apples, or by amination or transamination of aldehydes. Some amines are derived from tyramine (e. g., hordenine, synephrine, octopamine, dopamine and noradrenaline and others from tryptophan (serotonin, tryptamine, melatonin; The occurrence of these biologically active amines in fruits and vegetables could influence their concentrations in human serum.

8.5 Changes in Post Harvesting Stages including Storage

It has been recognized for many years that fruit continues to undergo chemical changes after harvest until finally spoilage occurs as it is attacked by fungi, yeasts, or bacteria. Since the eating quality changes with these reactions and the monetary value of the crop depends on it, a few fruits have been studied extensively, and a considerable body of knowledge has been built up through the years.

Pears and cherries have been studied less frequently, while some fruits have scarcely been investigated. Apples, pears, citrus fruits, and bananas are stored for variable lengths of time before they are consumed; and the changes which occur in them are significant economically. Cherrie, plums and berries of all kinds are more perishable and reach market soon after picking. If these fruits are kept for any length of time, they must be either canned or frozen.

The changes of fruit after harvest are numerous and include changes in

1. Respiration,
2. Carbohydrate,
3. Water content and
4. Organic acids and pH

At the time of harvest the respiration rate of apples and pears has sunk to a low level. However, soon after picking, the uptake of oxygen and production of carbon dioxide begin to speed up until finally a climax is reached, called the climacteric. It is followed by a steady decrease in respiratory

rate, often called senescence. Bananas are normally picked green and they show a climacteric as they ripen. Tomatoes and possibly other fruit also show this surprising change in respiration. Many changes occur in the carbohydrate fraction of fruit during ripening, during the climacteric and during senescence. Green fruit usually contains an abundance of starch, but is short on the soluble sugars that give ripe fruit its sweetness. On ripening, however starches decrease and sugars increase in concentration.

When fruit is picked and severed from the plant, water no longer flows into the fruit although the loss of water continues. Usually water cannot be taken in through the skin in apple storage one problem is to prevent water loss so that the fruit does not wither and decease in value. In dry atmospheres, and particularly at high temperatures, water loss is rapid. One of the most obvious changes in fruit is the alteration in texture. Apples in storage slowly soften and the rate depends on the temperature of the storage barn as well as the variety of the apple.

Pears are picked in the hard stage and held at low temperatures until required for ripening. On return to room temperatures, they develop “sleepiness” and instead of ripening satisfactorily they turn brown as they soften. In pears as in apples there is a change in the pectic substances during softening with a rapid drop in the amount of protopectin and a rise in soluble pectin. During cold storage the amount of protopectin increases. Changes in cellulose, hemicelluloses, and lignins have been followed in a small number of fruits and with a few varieties. Pears show a rapid rise in xylans and arabans and decrease in cellulose. Pears contain lignins in the stone cells and are unlike other fruits in this respect. Organic acids decrease in apple pulp and in pears during storage. Both of these fruits contain a allege number of organic acids in low concentration, a small amount of citric acid, and larger amounts of malic acid.

8.6 Browning Reactions and Preventive Methods

When fruit and vegetable tissues are injured in any way or cut and peeled during processing, a darkening of the tissues called the browning reaction sometimes occurs. This reaction has been extensively studied for a few fruits and vegetables but for most it has had little research. Some browning reactions are enzymatic and only occur in fresh living tissue or at least in tissues that still contain active enzymes. Thus when enzymes are denatured by heat or any other agent this reaction no longer occurs. When orange juice is concentrated, it often darkens with a deleterious effect not only on the appearance but also on the flavor. This browning reaction is nonenzymatic; it occurs at temperatures above those which denature most enzymes.

8.6.1 Enzymatic Browning

Enzymatic browning occurs in many tissues whenever they are injured. The injury can be the result of bruising, cutting, freezing, or disease. That part of the injured fruit or vegetable which is exposed to are undergoes a rapid darkening. Browning occurs through the effect of an oxidase on a catechol compound to form either a peroxide or hydrogen peroxide. The hydrogen peroxide then oxidizes some other compound, the chromogen, to form brown pigment.

According to some studies the enzymes in numerous fruits and found some evidence for the presence of an oxidase and catechol compound in the fruits that darken readily (apple, apricot, banana, cherry, grape, peach, pear, and strawberry), but no evidence of these enzymes in the fruits that do not darken readily (lemon, orange, lime, grapefruit, red currants, melon, pine apple and tomato).

All fruits susceptible to browning should be processed as quickly as possible. Heating destroys the enzymes responsible for the reaction; thus when fruit is canned or made into jams or jellies, the browning reaction stops as soon as the fruit is heated sufficiently high to denature the enzyme. The exact temperature necessary varies with enzyme, rate of heat, pH, and other factors. Deoxygenation and vacuum closing have also been used to diminish oxidation.

In the home preparation of fruits, pineapple juice and lemon juice have long been used to prevent browning. Pineapple juice has a relatively high percentage of sulfhydryl compounds which are active antioxidants while lemon juice contains both citric acid and ascorbic acid.

The pH has an important effect on the rapidity with which browning occurs. Acid dips are sometimes used to lower the pH and by this methods delay or retard browning.

8.6.2 Non enzymatic Browning

Three hypotheses have been suggested to explain non enzymatic browning.

- The browning reaction that occurs between carbohydrates and aminoacids results in the formation of brown pigments. It is known as the Millard reaction and is believed by many to explain the browning found in processed fruit.
- Ascorbic acid undergoes oxidation with the formation of a compound which produces brown pigment.
- Carbohydrates or carbohydrate acids decompose to furfuraldehyde or related compounds which then polymerize or react with nitrogen compounds to form brown pigment.

The temperature of storage, the amount of moisture, and the exposure of the fruit or fruit juice to oxygen either during processing or storage are influential in the development of browning.

Oxygen uptake either during processing or during storage has generally been shown to be a factor in browning. Some oxidation is apparently essential for the development of the reaction. For example, when the head space in canned orange juice is increased, the rate of browning is proportional to the increase in the space. Dried fruit is always exposed to oxygen during processing. Slight decreased in reducing sugars appear to occur in orange juice concentrates as browning occurs.

8.7 Changes in Cooking and Processing

The chemical changes that occur when fruits and vegetables are boiled in water, steamed, canned, dried or frozen are still for the most part unknown.

- As a result of changes in cellular structure, all fruits and vegetables undergo softening when cooked. The cells are separating, rupturing, shrinking, or that a combination of these changes occurs. When cells separate during processing, the cementing substances between the cells, in the middle lamella, must alter. Thus, since the principal compounds in the middle lamella are believed to be the pectin substances, their change is the first one considered.
- Since the Cell walls are known to be composed of a matrix of cellulose sometimes encrusted with other materials, the possibility of changes in cellulose must be considered.

- Starch granules can be seen in microscopic sections of most fruit and vegetable tissues and are known to swell rapidly in water.
- The intercellular air changes in volume when subjected to higher temperatures because of expansion
- Alterations in pigments, formation of acids and release of low molecular weight sulfur compounds are indicated from observations of gross changes.

The pectic substances are also of considerable importance in the firming of canned tomatoes, apples and other fruits by calcium salts. Canned tomatoes which are graded A and which therefore bring the highest price must be relatively firm, yet full coloured. However during ripening, tomatoes pass through this period rapidly, soften and on canning undergo considerable maceration and shredding. The addition of small amounts of calcium salts to the pack increases the firmness of the fruit. Calcium salts can be added to the dip or placed in the can with the salt.

The parenchyma tissue of fruits and vegetables is composed of cells separated by tiny pockets and passages of air. In some fruits and vegetables the amount of air may be appreciable, in others quite small. Fresh peaches may have as much as 15 percent air while plums have very little. When the fruit or vegetable undergoes processing, changes occur in th intercellular air. If the product is simply heated, the air will swell, force the cells apart, and often cause cracks in the food. Thus in a baked potato or baked apple, swelling occurs; however, on cooling, the product shrinks and cracks in the body are noticeable. Further changes may take place as the cell walls become more permeable. Cell sap, the solution held in the vacuoles, may escape into the intercellular spaces, causing a change in the appearance and juiciness of the food. Thus the chalky appearance of the product changes to one of translucence as the air is replaced with water.

This can be readily seen when green beans are placed in hot water. As soon as the bean is submerged, it begins to look greener because of the discharge of air from around the hairs on the surface and perhaps from between some of the surface cells. In many foods juiciness is markedly affected because the fluid that now fills the intercellular spaces readily escapes from tissues. On boiling a fruit or vegetable a third change may occur: the discharge of intercellular air and the filling of the spaces with the cooking water.

Many green vegetables contain volatile acids that are partially given off during cooking. These acids are of importance during cooking because they have a marked effect on the colour and flavor of the cooked vegetable. If a lid is placed on the cooing vessel, these acids dissolve in steam which condenses on the lid, drop back into the cooking water, and lower its pH. Chlorophyll, which is very sensitive to any pH below 7, will change to pheophytin and other olive green pigments. Green beans, spinach or broccoli cooked in a covered kettle will be browner in colour than another sample cooked without a lid for the same length of time and under the same conditions. When the lid is left off the vessel, these volatile acids are partially evaporated. They affect the flavor in two ways:

- They have sourness and flavor themselves
- They speed up hydrolysis of sulfur containing glycosides and produce distasteful sulfur compounds. The volume of water in which one of these green vegetables is cooked is likewise important since these volatile acids are readily soluble in water and can be diluted but using generous amount of cooking water.

8.8 Summary

The composition of vegetables can vary significantly depending upon the cultivar and origin. The amount of dry matter in most vegetables is between 10 and 20%. All fruits contain cellulose, hemicellulose (pentosans) and pectins. The building blocks of these polysaccharides are glucose, galactose, mannose, arabinose, xylose, rhamnose, fucose and galacturonic and glucuronic acids. The pectin fractions of fruits are particularly affected by ripening. It has been recognized for many years that fruit continues to undergo chemical changes after harvest until finally spoilage occurs as it is attacked by fungi, yeasts, or bacteria. Since the eating quality changes with these reactions and the monetary value of the crop depends on it, a few fruits have been studied extensively, and a considerable body of knowledge has been built up through the years.

When fruit is picked and severed from the plant, water no longer flows into the fruit although the loss of water continues. Usually water cannot be taken in through the skin in apple storage one problem is to prevent water loss so that the fruit does not wither and decease in value. When fruit and vegetable tissues are injured in any way or cut and peeled during processing, a darkening of the tissues called the browning reaction sometimes occurs. This reaction has been extensively studied for a few fruits and vegetables but for most it has had little research.

As a result of changes in cellular structure, all fruits and vegetables undergo softening when cooked. The cells are separating, rupturing, shrinking, or that a combination of these changes occurs. Starch granules can be seen in microscopic sections of most fruit and vegetable tissues and are known to swell rapidly in water. The parenchyma tissue of fruits and vegetables is composed of cells separated by tiny pockets and passages of air. In some fruits and vegetables the amount of air may be appreciable, in others quite small. Fresh peaches may have as much as 15 percent air while plums have very little. When the fruit or vegetable undergoes processing, changes occur in th intercellular air. If the product is simply heated, the air will swell, force the cells apart, and often cause cracks in the food. Thus in a baked potato or baked apple, swelling occurs; however, on cooling, the product shrinks and cracks in the body are noticeable.

8.9 Self Assessment Questions

1 What is the composition of various fruits and vegetables?
2 What is the chemistry of fruits and vegetables?
3 Explain the changes in post harvesting stages of fruits and vegetables
4 How to prevent the browning of fruits and vegetables?
5 Explain in detail about enzymatic and non enzymatic browinig?
6 What are the various changes takes place during cooking of vegetables?

8.10 Reference Books:

1 H.D.Belitz, W.Grosch and P.Schieber. Food Chemistry 4th revised and extended edition 2009
2 John M. deMan, Principles of food chemistry, Third edition, 1999
3 Upadhyay and Upadhyay, Biophysical chemistry, 2006
4 Albert L. Lehninger and David L.Nelson Principles of Biochemistry, 5th edition
5 Lillian Hoagland Meyer, Food chemistry, Litton Educational Publishing inc, U.S.A.

Dr.P. KIRANMAYI

Lesson - 9

WATER INSOLUBLE PLASTID PIGMENTS

9.0 Objective

After going through this lesson you will learn:

- The chemical structure of carotenoids
- Changes in carotenoids during cooking
- Chemical structure of chlorophylls

Structure

9.1 Introduction

The colour of fruits and vegetables is exceedingly important to our pleasure at the table. It is not only the subtle variety of flavours that makes fruits and vegetables so pleasing, but also their variety of delicate and bright colours. Most of the pigments occur in plastids, specialized bodies lying in the protoplasm of the cell. For example, the chlorophylls occur in the chloroplasts, which under the microscope can be seen next to the cell wall as distinct bodies containing flecks of green pigment. Occasionally a pigment is present in the protoplasm as a crystal. Thus in carrot cells. platelets of carotene can be observed and in tomatoes, needles or platelets of lycopene. Sometimes the water soluble pigments are dissolved in the vacuoles, and not generally distributed through the cell.

The chief pigments of fruits and vegetables can be classified as

1. The carotenoids, 2. The chlorophylls, 3. The anthoxanthins and 4. The anthocyanins. Tannins often account for the formation of off-colours during processing.

9.2 The Carotenoids

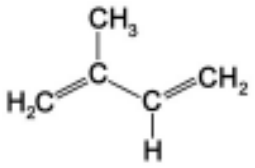
9.2.1 Chemical structure and changes in cooking

The carotenoids are a group of yellow, orange and orange-red fat soluble pigments widely distributed in nature. In green leaves they occur in the chloroplasts, small bodies close to the cell

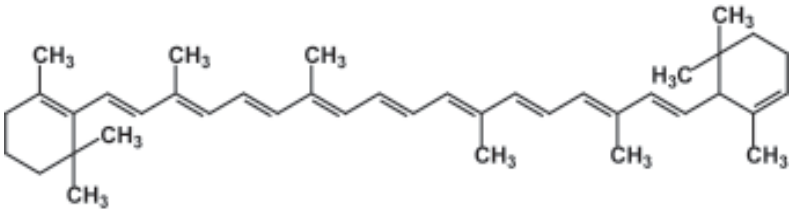
walls of the palisade cells. These cells are next to the epidermal cells on the upper side of the leaf. The carotenoids are present in the lipid material along with the chlorophylls.

The green colour of the chlorophyll masks the yellow to red colour of the carotenes except in very young leaves while the amount of chlorophyll is small. The bright, fresh yellow-green colour of spring leaves is the result of carotenoids and small amounts of chlorophylls. These pigments are also present in a wide variety of fruits peaches, banana skins, tomatoes, red peppers, paprika, rose hips, squash etc. as well as other parts of plants in carrots, sweet potatoes, and in most yellow, orange, and red flowers. When they are consumed by animals, they tend to concentrate in lipids and hence are found in blood, milk, egg yolk and depot fat.

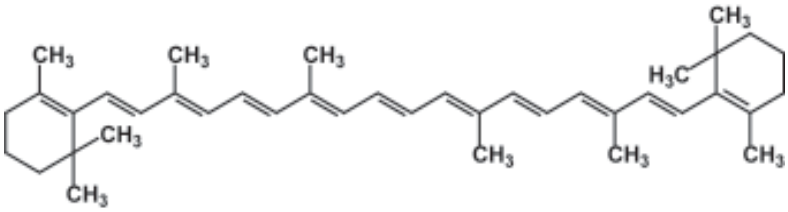
The name carotenoid is applied to all pigments chemically related to the carotenes, which were the first isolated in 1831, the carotene is a mixture of three isomers, á, â, and ã carotene. The carotenoids are either hydrocarbons or derivatives of hhydrocarbons and are composed of isoprene units. Isoprene is a diene, and this molecule is the unit out of which the carotenoids are constructed. It contains five carbon atoms while many, although not all, of the carotenoids contain 40 carbon atoms or eight isoprene units. â-carotene and lycopene are examples of symmetrical molecules.



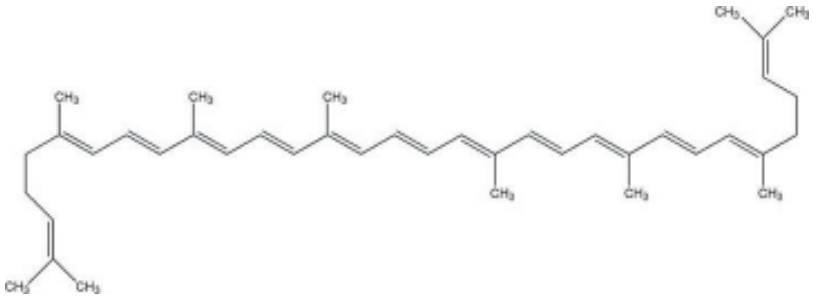
Isoprene



á- Carotene



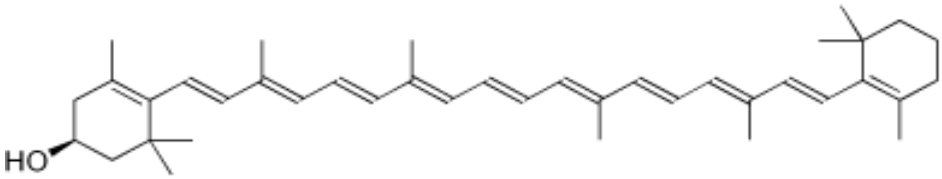
â – carotene



Lycopene

The carotenoids are insoluble in water but soluble in lipids and in lipid solvents. In processing fruits and vegetables, loss of these pigments into cookery or canning water is very slight. They do undergo oxidation when exposed to air, so that in drying fruits or vegetables which contain these pigments a problem is sometimes encountered. For example, carrots and apricots show loss of pigment on drying. These pigments do not undergo hydrolysis except when they occur in plant tissues as esters, and they are not affected by changes in pH.

Carotenoids which contain hydroxyl groups are called xanthophylls. A number of xanthophylls have been isolated from plants and their structures determined partially or completely. They are often associated with carotenes; leaves contain not only the hydrocarbon carotenes as their yellow pigments but also the closely related xanthophylls. Cryptoxanthin is an example of the xanthophylls.



Cryptoxanthin

In blanched carrots, or those blanched and then dehydrated, the loss of pigments, is rapid. Carrots diced small and stored at 62^0C in moist air after blanching show a complete loss of pigment in 20 hours. The carotenoids in carrots are present in the chromoplasts. In some cells starch is present and the granules may more or less surround the carotene crystals. In the outer cells or of the root the carotene concentration is highest, there is little or no starch, and the carotene is present in crystals as needles, tubes, flakes, or spirals. These cells also contain tiny fat droplets. When the cell is killed by blanching, drying or chemical reagents, the chromoplasts disintegrate and the carotene dissolves in oil droplets. The oil droplets are sometimes originally present in the living cell or they may separate from the protoplasm after it is killed.

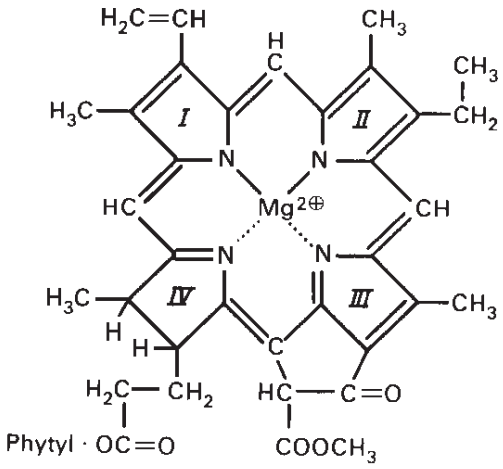
Carrot	á-carotene, â-carotene, ã-carotene, xanthophylls
Wheat germ	Xanthophyl, carotene
Corn	Zeaxanthin, cryptoxanthin, xanthophylls, á-carotene, â-carotene, ã-carotene, neocryptoxanthin, hydroxyl-á-carotene
Apricot	â-carotene, ã-carotene, lycopene
Peach	á-carotene
Soya bean	á-carotene, â-carotene
Cow pea	â-carotene, xanthophylls
Orange	Zeaxanthin, â-carotene, lycopene, cryptoxanthin, xanthophylls, violaxanthin, â-citraurin
Grape fruit	â-carotene, lycopene
Squash	á-carotene, â-carotene, xanthophylls, violaxanthin
Red pepper or chili	á-carotene, â-carotene, capsanthin

Distribution of carotenoids in some foods

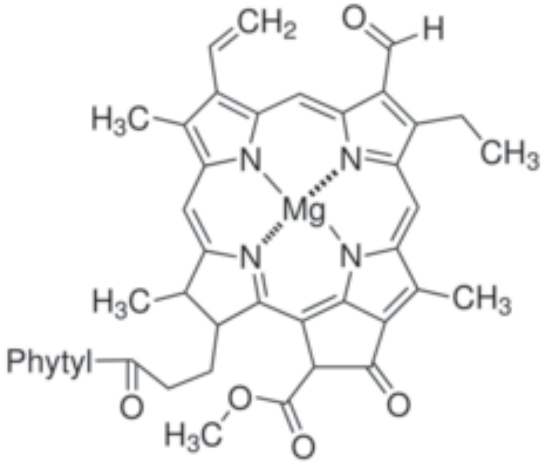
9.3 Chlorophylls

9.3.1 Chemical structure and changes in cooking

The green pigments of leaves and stems are usually held close to the cell wall in small bodies, the chloroplasts, along with some carotenes and xanthophylls. Two chlorophylls have been isolated, chlorophyll a and chlorophyll b; and they occur in plants in the ratio of approximately 3a:1b. Chemically, they are very similar. They belong to that group of important biological pigments the porphyrins, which includes hemoglobin. They are fairly large molecules composed of four pyrrole rings held together by methane carbons (-CH=) to form a large flat molecule. In chlorophyll, a magnesium atom is held by the nitrogen on two of the rings by ordinary covalent bonds. The other two nitrogens share two electrons with the magnesium to form a coordinate covalent bond. The formulas for chlorophyll a and chlorophyll b are given below:

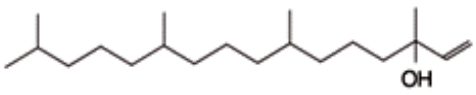


Chlorophyll *a*



Chlorophyll b

The chlorophyll a is an ester. The propionic residue on position 7 is esterfied with phytol, while the acid joined to position 6 is esterfied with methanol. Phytol, or phytyl alcohol, is an interesting high molecular weight acohol because it occurs as an ester in a number of plant molecules. It has a chain which, although almost completely saturated, has an arrangement of carbons very similar to that in carotenoids.



Phytol

Chlorophyll b differs in the occurrence of a –CHO (formyl) group in position 3 in place of the methyl group (-CH_3) in chlorophyll a. The chlorophylls are of great importance in the plant because of their role in photosynthesis and the formation of carbohydrates from carbondioxide and water. Chlorophyll changes to an olive green colour and then to brown. The most likely reaction is the substitution of hydrogen for the magnesium which has been complexed in the porphyrin to form pheophytin. It is unlikely that hydrolysis of either of the esters can be involved. Ester groups usually hydrolyze slowly and this reaction of chlorophyll occurs rapidly. Also the reaction is rapid in acid solutions and does not occur readily in cooking waters where the pH is 8 or more.

Esters, on the other hand, are more readily hydrolyzed in alkaline solutions than in acid. The possibility of oxidation as the reactions is likewise small since the brown colour develops therefore, that when a vegetable becomes olive green on cooking, the chlorophyll has formed pheophytin.

When green beans are first dropped into boiling water, they like other green vegetables, show a change in colour. The green brightens, the velvety appearance disappears, and the beans become more translucent. These changes are probably caused first by the wetting of the fine hairs on the coat of the bean. Washing the bean and rubbing it produce the same results to the slight degree. Then as the bean is warmed, air is expelled and the intercellular spaces collapse or partially collapse. As cooking continues, plant acids are liberated; and because the chlorophyll is released from the protein complex or because the chlorophyll is released from the protein complex or because the membrane around the chloroplasts becomes more permeable or both, the acids react with the chlorophylls and form pheophytin.

When green vegetables such as spinach or cabbage, which produce considerable volatile acid during the early part of cooking, are cooked in a pot with a cover, the colour very quickly changes to olive green and then to a dull brown. But if the lid is left off so that acids escaped during the early part of cooking colour retention is much better. Since it is possible to maintain the green colour in the presence of alkali you might wonder why it is always recommended that baking soda, sodium bicarbonate, be omitted in cooking vegetables. With a high pH in the cooking or canning water, particularly ascorbic acid and thiamine, are very sensitive to heating at high pH's and in cooking water to which sodium bicarbonate has been added, the rate of destruction of these vitamins is accelerated.

Some metal ions react with the chlorophylls to form compounds with bright green colours. Ferric, zinc and cupric ions will replace the magnesium in chlorophyll. They will also react with the pheophytins which have formed from the reactions of the chlorophylls with plant acids and produce the corresponding complex. Green leaves boiled for 1 or 2 minutes, or frozen and then thawed. After fresh mallow leaves are boiled, there is a reduction in the amounts of chlorophyll a and b and the appearance of chlorophyll a' and b', isomers chlorophyll a and b. leaves frozen and then thawed yielded no other green pigments, but there was evidence that neither the oxidative nor the hydrolytic enzymes are inactivated. On drying the leaves in air for 24 hrs at 20^0C chlorophyll a and b formed not only chlorophyll a' and b', but pehophytins as well. The enzymes are inactivated on drying.

9.4 Summary

Most of the pigments occur in plastids, specialized bodies lying in the protoplasm of the cell. For example, the chlorophylls occur in the chloroplasts, which under the microscope can be seen next to the cell wall as distinct bodies containing flecks of green pigment. The carotenoids

are a group of yellow, orange and orange-red fat soluble pigments widely distributed in nature. In green leaves they occur in the chloroplasts, small bodies close to the cell walls of the palisade cells. These cells are next to the epidermal cells on the upper side of the leaf. The carotenoids are insoluble in water but soluble in lipids and in lipid solvents. In processing fruits and vegetables, loss of these pigments into cookery or canning water is very slight. They do undergo oxidation when exposed to air, so that in drying fruits or vegetables which contain these pigments a problem is sometimes encountered.

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9.5 Self Assessment Questions

1 What are the various changes takes place in carotenoids while cooking?
2 What is the chemical structure of chlorophyll?
3 Explain about changes takes places in chlorophyll while cooking.

9.6 Reference Books:

1. H.D.Belitz, W.Grosch and P.Schieber. Food Chemistry 4th revised and extended edition 2009
2. John M. deMan, Principles of food chemistry, Third edition, 1999
3. .Upadhyay and Upadhyay, Biophysical chemistry, 2006
4. Albert L. Lehninger and David L.Nelson Principles of Biochemistry, 5th edition
5. Lillian Hoagland Meyer, Food chemistr, Litton Educational Publishing inc, U.S.A.

Dr .P. KIRANMAYI

LESSON - 10

WATER SOLUBLE PIGMENTS

10.0 Objective

After going through this lesson you will learn:

- The structure of anthocyanins
- The structure of Anthoxanthine and Flavones

Structure

10.1 Introduction

The flavonoids are a group of compounds widely distributed in the plant kingdom. They are water soluble and are often present in the juices of plants. Chemically the favonoids contain two benzene rings with a three carbon bridge. In most the three carbon bridge is condensed through an oxygen into an intermediate ring. The benzene rings hold hydroxyl groups. The true flavonoids consist of the anthocyanins which are the red-blue-purple pigments of plants, the anthoxanthins which are yellow; the catchins; and the leucoanthocyanins. The last two groups of compounds are colourless but readily change to brownish pigments. They are probably the so called “food tannins”.

10.2 Anthocyanins

Most of the red, blue, and violet pigments that occur in flowers, fruits and other parts of plants belong to the group of pigments known as anthocyanins. These occur in plant cells as glycosides which are ethers of monosaccharide moiety and sometimes with two. The colour results from the strucuture of the anthocyanidin which is combined with the monosaccharides. The carbohydrates commonly bonded to the anthocyanidins are glucose, galactose, rhamnaoe, and occasionally a pentose. Most of the anthocyanins are soluble in water, and it is only on boiling with fairly concentrated mineral acid, a condition which is not encountered in food preparation, that the pigments are hydrolyzed to form the anthocyanidin and the carbohydrate.

Anthocyanin → Anthocyanindin + Manose

Only three types of anthocyanidins have been identified in plant tissues, although a number of methyl derivatives of these three have been isolated. Pelargonidin, cyanidin and delphinidin are wide spread in nature, with cyanidin the most common. The great variety of colours, hues and tints that occur in nature and the subtle shading on the cheek of fruit or in a blossom are the result of a number of factors.

At low pH: these pigments are red; the hues may be different but they are all reddish. Thus pelargonidin is orange-red in acid solution while delphinidin is a bluish red. At high pH's the anthocyanins pass through a violet and then blue colour. Some turn green and then yellow at very high pH's. However, in the red rose the pigment occurs as a result of an acid while in the blue corn flower it is present as metal salts. corn flowers also contain a flavones, apigenin, which is colorless but causes blueing of cyanin.

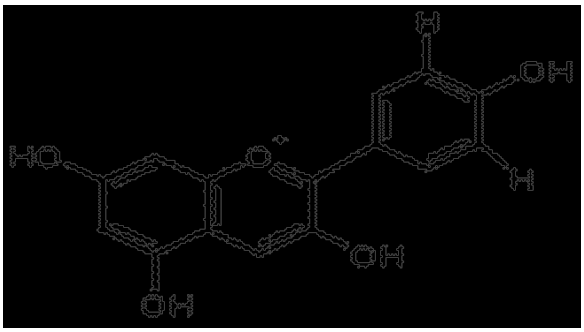
The concentration of the pigment alters the hue: when an acid solution of synthetic delphinidin is poured on filter paper a dilute solution gives a blue colour while a concentrated solution gives red. An intermediate concentration gives a purple colour. When tannins are present this is more pronounced.

In the cell sap: the anthocyanins are frequently adsorbed on colloidal particles, probably polysaccharides. The pH is stabilized and the colour influenced by this association. In blue corn flowers cyanin is adsorbed and the pH stabilized at 4.9

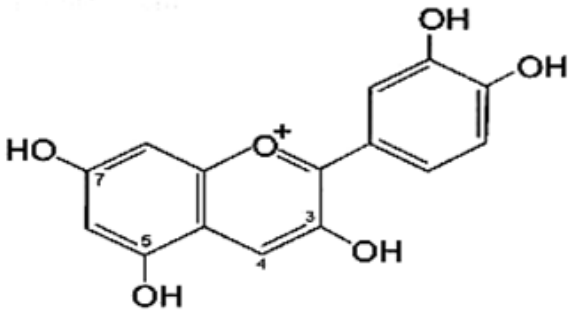
Frequently the anthocyanins occur as mixtures: as the composition of the mixture is altered the hue changes. Thus blue grapes contain not only glucosides of delphinidin but also of syringidin, the dimethyl ether of delphinidin.

Sometimes plant cells will contain: not only the anthocyanins as pigment but also some of the anthoxanthins which may be yellow and often yellow carotenoids.

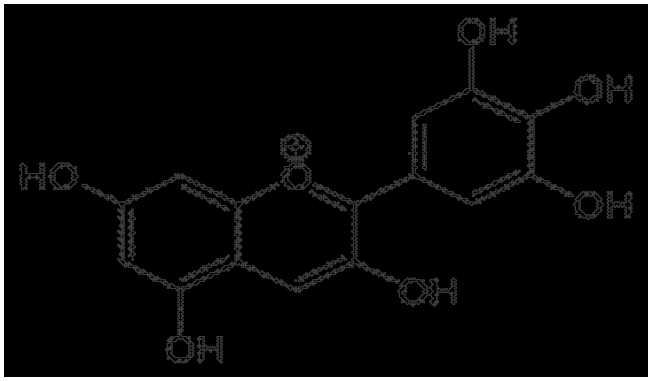
- Tannins are often associated with anthocyanins and alter the colour



Pelargonidin



cyanidin



Delphinidin

Fruits and vegetables which contain the anthocyanins, the red and violet foods, present a problem in cookery and processing because of the great solubility in water of the pigments. There is always a tendency for the pigment to leach out in the cooking or canning water or to run out in the juice. However, if the cell walls remain intact the pigment is not lost. Thus frozen red raspberries show excellent retention of the pigment, but in canned berries the colour gradually passes out into the canning solution until berries are practically colourless.

The effect of changes of pH on the colour of the anthocyanins is often noticed in food preparation. Most fruits contain sufficient acid so that the pigment remains red or bluish red through the cookery or processing. When vinegar is added to beets in pickling, the colour often reddens. When red cabbage is cooked in soft water with a pH near 7, there is a bluing of the colour. If vinegar is added to blue cabbage, the colour will change back to red.

The anthocyanins form salts with metal ions, and have colours that depend not only on the particular anthocyanin but also on the metal ion. Most of the colours are grayish purple. The

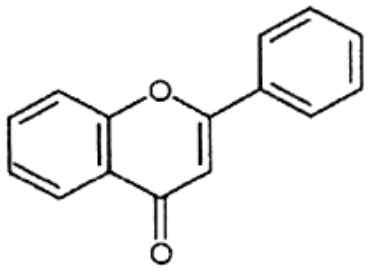
reaction is particularly important in caning and often in cookery. When tin cans are used for canning hose fruits or vegetables containing anthocyanins, it is necessary to lacquer the inside of the can. Tin cans without this lacquer will cause a discolouration of the fruit touching the side of the can.

If a tin pie plate is used for preparing blue berry pie, the bottom of the crust becomes a grayish blue colour. This is particularly noticeable if the tin has been scratched so that some of the iron is exposed. Small deposits of rust form, the fruit acids rapidly dissolve the rust, and the amount of reaction with the anthocyanins is increased. If a cherry pie is cut with a steel knife and the knife is allowed to remain in contact with the juice, the change to purple is observed. When aluminum salts are added to fruit juices which contain the anthocyanins, a change in colour occurs. But it is usually not as marked as with iron. In cooking fruits for jams and jellies in aluminum vessels some of the change in colour may be the result of the reaction with aluminum ions. Iron vessels cause a marked alteration in the colour of the fruit and must be avoided.

Prolonged storage of fruits with red or red-violet pigments is accompanied by bleaching of some pigment and the development of red-brown and finally a brown colour. Storage temperature is most important in the rate at which the change in colour occurs. Ascorbic acid protects the pears, plums, and peaches against discolouration and the development of off-flavours, but it does not protect grape juice.

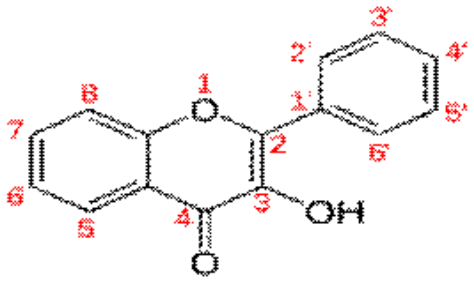
10.3 Anthoxanthins and Flavones

One of the most important groups of pigments in plants are the anthoxanthins and the flavones. They are yellow pigments usually dissolved in the cell sap. The anthoxanthins are glycosides which on boiling with dilute acid yield one or two molecules of monosaccharides and a flavones or a flavones derivative such as3a flavonal, flavanonal, or isoflavone. The basic ring structure of the flavones is

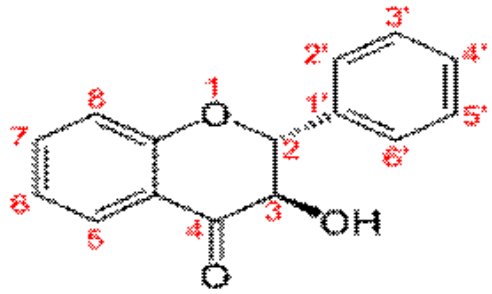


Flavone

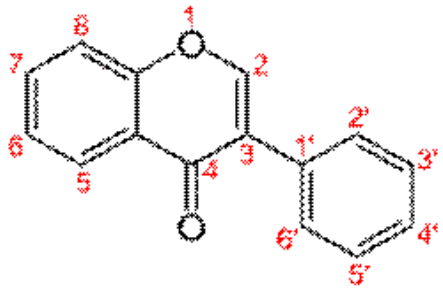
Flavanols have a hydroxyl group in positon 3; flavononols do not have a double bond between carbons 2 and 3; and isoflavones have the phenyl group in position 3 instead of 2. Most flavones contain a number of hydroxyl groups and some contain methoxyls. In the anthoxanthins one or more hydroxyls.



Flavonol



Flavanonol



Isoflovone

These pigments occur dissolved in the cell sap and are usually pale yellow or colourless but occasionally bright orange. Most bright yellow or orange fruits and vegetables are coloured by carotenoids rather than anthoxanthins and flavones. Examples of some of the flavones which occur in this group are: Quercetin occurs in onion skins, tea, hops, horse chest nut, sumach, red rose and the bark of the American oak and many other tissues. This glycosides are likewise widely distributed. The anthoxanthins are yellow to orange in colour and are dissolved in the cell sap of flowers, stems, leaves, roots and even wood. They are water soluble pigments and differ in this from the carotenoids, the other group of yellow pigments, which are lipid soluble.

When food is cooked in alkaline waters we often see the development of in alkaline waters we often see the development of a yellow or cream colour. Hard water will often have a pH as high as 8 and softened water which contains $NaHCO_3$ in place of $Ca(HCO_3)$ will have a pH even higher.

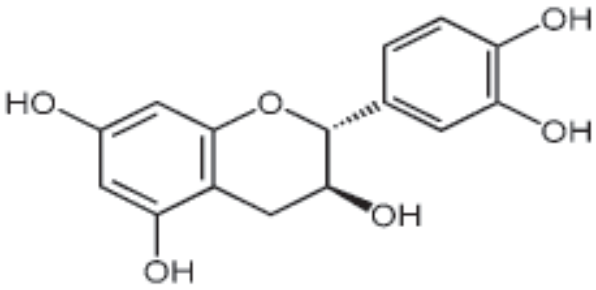
Potatoes cooked in softened water often have a creamy colour. The occurrence of this colour can be prevented or the colour removed by adding a little cream of tartar. If the potatoes are cooked in chunks, band as yellow are sometimes seen where the pigment is more concentrated in some cells. Rice also shows this yellowing when cooked in softened water, and it too can be kept a clear, bright white by adding cream of tartar to the cooking water. This effect is most noticeable in onions, particularly yellow skinned, for the flesh turns pale yellow and the cooking water is bright yellow when alkaline water is used. Cauliflower and cabbage likewise sometimes show a yellowing. Occasionally cauliflower turns a brown or pinkish brown color on cooking. Part of the colour may be caused by flavones, but most is the result of reactions of ions with the tannins. The change colour has probably most frequently been noticed when the acid of lemon juice causes a fading the colour.

Many of the flavones have definite physiological effects on man. Indeed the active principle of some old drugs, used long ago, has been shown to be either one or a mixture of flavones or anthoxanthins. On eof the most interesting is the mixture which has been called vitamin P or citrin.

10.4 Tannins

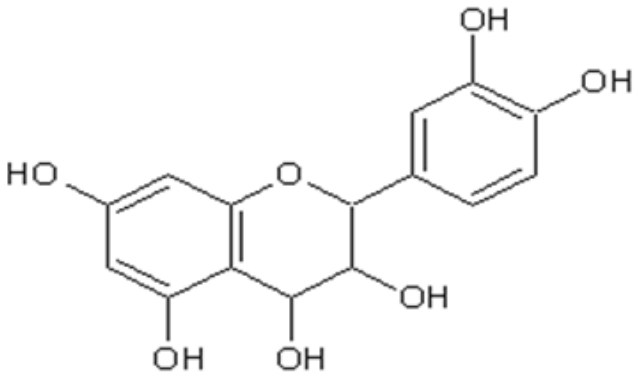
Some plant substances are able to react with components in the skins of animals and “tan” them. The leather produced is much more durable than the dried skin. The tannins react with a number of ions and form dark colours which have been used for inks; they are readily oxidized with permanganate and can be titrated.

Today the tannins of foods appear to be comprised of catechins, the lucoanthocyanins, and some hydroxyl acids. All of them give colours with metal ions. Those which are ortho and para dihydroxy benzene derivatives are readily oxidized by permanganate although the monohydroxy of meta dihydroxy derivatives are not. The substances that react with the proteins in skins and bring about tanning are probably polymers of catechins with intermediate molecular weights. The low molecular weight compounds in many fruits and vegetables are related to them.



Catechin

Catechin and epicatechin are reduced derivatives of flavones. They are isomers in which the ring and hydroxyl are probable trans in catechin and cis in epicatechin. The structure of the leucoanthocyanins is still uncertain but they are probably closely related to the catechins. Catechins and leucoanthocyanins are present in the tissues of those woody plants studies such as apples, peaches, grapes, almonds, and some pears, while they are absent in herbaceous plants. They are present in cereals although the amounts vary.



Leucoanthocyanin

The tannins are readily dispersed in hot water, some in cold, to form colloidal systems. When a fruit is pressed as apples in the preparation of cider or grapes in making juice or wine, the tannins flow out in the juice. In extraction such as the brewing of tea or coffee some of the tannins are extracted. When the tea and coffee are brewed with hard water, a brown or red brown precipitate forms on the surface of the liquid; and as the beverage cools, it appears throughout the liquid.

10.5 Summary

The flavonoids are a group of compounds widely distributed in the plant kingdom. They are water soluble and are often present in the juices of plants. Chemically the favonoids contain two benzene rings with a three carbon bridge. The true flavonoids consist of the anthocyanins which are the red-blue-purple pigments of plants, the anthoxanthins which are yellow; the catchins; and the leucoanthocyanins. Most of the red, blue, and violet pigments that occur in flowers, fruits and other parts of plants belong to the group of pigments known as anthocyanins. These occur in plant cells as glycosides which are ethers of monosaccharide moiety and sometimes with two.

Fruits and vegetables which contain the anthocyanins, the red and violet foods, present a problem in cookery and processing because of the great solubility in water of the pigments. There is always a tendency for the pigment to leach out in the cooking or canning water or to run out in the juice. Some plant substances are able to react with components in the skins of animals and “tan” them. The leather produced is much more durable than the dried skin. The tannins react with a number of ions and form dark colours which have been used for inks; they are readily oxidized with permanganate and can be titrated.

10.6 Self Assessment Questions

1. What are the water soluble pigments and their chemical structure?
2. Explain about anthocyanins and how they are changed during cooking.
3. Discuss about structure of tannins and how they are changed during cooking.

10.7 Reference Books

1. H.D.Belitz, W.Grosch and P.Schieber. Food Chemistry 4th revised and extended edition 2009
2. John M. deMan, Principles of food chemistry, Third edition, 1999
3. Upadhyay and Upadhyay, Biophysical chemistry, 2006
4. Albert L. Lehninger and David L.Nelson Principles of Biochemistry, 5th edition
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Dr .P. KIRANMAYI

Lesson - 11

FOOD ENZYMES

11.0 Objective

After going through this lesson you will learn:

- Nature and functions of enzymes
- Types of enzymes

Structure

11.1 Introduction

Enzymes, although minor constituents of many foods, play a major and manifold role in foods. Enzymes that are naturally present in foods may change the composition of those foods; in some cases, such changes are desirable but in most instances are undesirable, so the enzymes must be deactivated. The blanching of vegetables is an example of an undesirable change that is deactivated. Some enzymes are used as indicators in analytical methods; phosphatase, for instance, is used in the phosphatase test of pasteurization of milk. Enzymes are also used as processing aids in food manufacturing. For example, rennin, contained in extract of calves' stomachs, is used as a coagulant for milk in the production of cheese. Food science's emphasis in the study

of enzymes differs from that in biochemistry. The former deals mostly with decomposition reactions, hydrolysis, and oxidation; the latter is more concerned with synthetic mechanisms.

11.2 Nature and Function

Enzymes are proteins with catalytic properties. The catalytic properties are quite specific, which makes enzymes useful in analytical studies. Some enzymes consist only of protein, but most enzymes contain additional non protein components such as carbohydrates, lipids, metals, phosphates, or some other organic moiety. The complete enzyme is called holoenzyme; the protein part, apoenzyme and the non protein part, cofactor. The compound that is being converted in an enzymic reaction is called substrate. In an enzyme reaction, the substrate combines with the holoenzyme and is released in a modified form.

11.3 Types of Enzymes

11.3.1 Hydrolases

The hydrolases as a group include all enzymes that involve water in the formation of their products. For a substrate AB, the reaction can be represented as follows:

$$AB + HOH \rightarrow HA + BOH$$

The hydrolases are classified on the basis of the type of bond hydrolyzed. The most important are those that act on ester bonds, glycosyl bonds, peptide bonds, and C-N bonds other than peptides.

11.3.2 Esterases

The esterases are involved in the hydrolysis of ester linkages of various types. The products formed are acid and alcohol. These enzymes may hydrolyze triglycerides and include several lipases; for instance, phospholipids are hydrolyzed by phospholipases, and cholesterol esters are hydrolyzed by cholesterol esterase. The carboxylesterases are enzymes that hydrolyze triglycerides such as tributyrin. They can be distinguished from lipases because they hydrolyze soluble substrates, whereas lipases only act at the water lipid interfaces of emulsions. Therefore, any condition that results in increased surface area of the water-lipid interface will increase the activity of the enzyme. This is the reason that lipase activity is much greater in homogenized (not pasteurized) milk than in the non homogenized product.

11.3.3 Amylases

The amylases are the most important enzymes of the group of glycoside hydrolases. These starch-degrading enzymes can be divided into two groups, the so-called debranching enzymes that specifically hydrolyze the 1, 6-linkages between chains, and the enzymes that split the 1, 4 - linkages between glucose units of the straight chains.

11.3.4 Pectic Enzymes

The pectic enzymes are capable of degrading pectic substances and occur in higher plants and in microorganisms. The presence of pectic enzymes in fruits and vegetables can result in excessive softening. They are not found in higher animals, with the exception of the snail.

11.3.5 Proteases

Proteolytic enzymes are important in many industrial food processing procedures. The reaction catalyzed by proteolytic enzymes is the hydrolysis of peptide bonds of proteins. Proteolytic enzymes can be divided into the following four groups: the acid proteases, the serine proteases, the sulfhydryl proteases, and the metal-containing proteases.

11.3.5.1 Acid Proteases

This is a group of enzymes with pH optima at low values. Included in this group are pepsin, rennin (chymosin), and a large number of microbial and fungal proteases.

11.3.5.2 Serine Proteases

This group includes the chymotrypsins, trypsin, elastase, thrombin, and subtilisin. The name of this group of enzymes refers to the seryl residue that is involved in the active site. The chymotrypsins, trypsin and elastase, are pancreatic enzymes that carry out their function in the intestinal tract. They are produced as inactive zymogens and are converted into the active form by limited proteolysis.

11.3.5.3 Sulfhydryl Proteases

These enzymes obtain their name from the fact that a sulfhydryl group in the molecule is essential for their activity. Most of these enzymes are of plant origin and have found widespread use in the food industry. The only sulfhydryl proteases of animal origin are two of the cathepsins, which are present in the tissues as intracellular enzymes.

11.3.5.4 Metal-Containing Proteases

These enzymes require a metal for activity and are inhibited by metal-chelating compounds. They are exopeptidases and include carboxypeptidase A (peptidyl-L-amino-acid hydrolase) and B (peptidyl-L-lysine hydrolase), which remove amino acids from the end of peptide chains that carry a free á-carboxyl group.

11.3.6 Oxidoreductases

Phenolases

The enzymes involved in enzymatic browning are known by the name polyphenoloxidase and are also called polyphenolase or phenolase. The action of polyphenolases is detrimental when it leads to browning in bruised and broken plant tissue but is beneficial in the processing of tea and coffee.

Catalase

Catalase catalyzes the conversion of two molecules of hydrogen peroxide into water and molecular oxygen as follows:

$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2$$

Catalase is less stable to heat than is peroxidase. At neutral pH, catalase will rapidly lose activity at 35^0C.This enzyme occurs in plants, animals, and microorganisms.

Peroxidase

The reaction type catalyzed by peroxidase involves hydrogen peroxide as an acceptor, and a compound AH_2 as a donor of hydrogen atoms, as shown:

$$H_2O_2 + AH_2 \xrightarrow{\text{peroxidase}} 2\,H_2O + A$$

In contrast to the action of catalase, no molecular oxygen is formed. it plays an important role in the development and senescence of plant tissues.

Lipoxygenase

This enzyme, formerly named lipoxidase, is present in plants and catalyzes the oxidation of unsaturated fats. The major source of legumes, soybeans, and other beans and peas. Smaller amounts are present in peanuts, wheat, potatoes, and radishes. Addition of soybean flour to wheat flour dough results in a bleaching effect, because of oxidation of the xanthophyll pigments. In addition, there is an effect on the rheological and baking properties of the dough. It has been suggested that lipoxygenase acts indirectly in the oxidation of sulfhydryl groups in the gluten proteins to produce disulfide bonds. When raw soybeans are ground with water to produce soy milk, a strong and unpleasant flavor develops that is called painty, green, or beany. Carrying out the grinding in boiling water instantly deactivates the enzyme, and no off-flavor is formed.

Xanthine Oxidase

This enzyme catalyzes the conversion of xanthine and hypoxanthine to uric acid. The xanthine oxidase in milk is associated with the fat globules and, therefore, follows the fat into the cream when milk is separated.

Uses of enzymes in food processing

Enzyme	*Food*	*Purpose or Action*
Amylases	Baked goods	Increase sugar content for yeast fermentation
	Brewing	Conversion of starch to maltose for fermentation; removal of starch turbidities
	Cereals	Conversion of starch to dextrins, sugar; increase water absorption
	Chocolate-cocoa	Liquidification of starches for free flow
	Confectionery	Recovery of sugar from candy scraps
	Fruit juices	Remove starches to increase sparkling properties
	Jellies	Remove starches to increase sparkling properties
	Pectin	An aid in preparation of pectin from apple pomace
	Syrups and sugars	Conversion of starches to low molecular weight dex trins (corn syrup)
	Vegetables	Hydrolysis of starch as in tenderization of peas
Cellulase	Brewing	Hydrolysis of complex carbohydrate cell walls
	Coffee	Hydrolysis of cellulose during drying of beans
	Fruits	Removal of graininess of pears; peeling of apricots,, tomatoes
Dextran-sucrase	Sugar syrups	Thickening of syrup
	Ice cream	Thickening agent, body
Invertase	Artificial honey	Conversion of sucrose to glucose and fructose
	Candy	Manufacture of chocolate-coated, soft, cream candies
Lactase	Ice Cream	Prevent crystallization of lactose, which results in grainy, sandy texture
	Feeds	Conversion of lactose to galactose and glucose
	Milk	Stabilization of milk proteins in frozen milk by removal of lactose
Tannase	Brewing	Removal of polyphenolic compounds
Pentosanase	Milling	Recovery of starch from wheat flour
Naringinase	Citrus	Debittering citrus pectin juice by hydrolysis of the glucoside, naringin
Pectic enzymes (useful)	Chocolate-cocoa	Hydrolytic activity during fermentation of cocoa
	Coffee	Hydrolysis of gelatinous coating during fermentation of beans
	Fruits	Softening
	Fruit juices	Improve yield of press juices, prevent cloudiness, improve concentration processes
	Olives	Extraction of oil
	Wines	Clarification

Enzyme	*Food*	*Purpose or Action*
Pectic enzymes (deteriorative)	Citrus juice	Destruction and separation of pectic substances of juices
	Fruits	Excessive softening action
Proteases (useful)	Baked goods	Softening action in doughs; cut mixing time, increase extensibility of doughs; improvement in grain, texture, loaf volume; liberate β-amylase
	Brewing	Body, flavor and nutrients development during fermentation; aid in filtration and clarification, chill-proofing
	Cereals	Modify proteins to increase drying rate, improve product handling characteristics; manufacture of miso and tofu
	Cheese	Casein coagulation; characteristic flavors during aging
	Chocolate-cocoa	Action on beans during fermentation
	Eggs, egg products	Improve drying properties
	Feeds	Use in treatment of waste products for conversion to feeds
	Meats and fish	Tenderization; recovery of protein from bones, trash fish; liberation of oils
	Milk	In preparation of soybean milk
	Protein hydrolysates	Condiments such as soy sauce and tamar sauce; specific diets; bouillon, dehydrated soups, gravy powders, processed meats
	Wines	Clarification
Proteases (deteriorative)	Eggs	Shelf life of fresh and dried whole eggs
	Crab, lobster	Overtenderization if not inactivated rapidly
	Flour	Influence on loaf volume, texture if too active
Lipase (useful)	Cheese	Aging, ripening, and general flavor characteristics
	Oils	Conversion of lipids to glycerol and fatty acids
	Milk	Production of milk with slightly cured flavor for use in milk chocolate
Lipase (deteriorative)	Cereals	Overbrowning of oat cakes; brown discoloration of wheat bran
	Milk and dairy products	Hydrolytic rancidity
	Oils	Hydrolytic rancidity
Phosphatases	Baby foods	Increase available phosphate
	Brewing	Hydrolysis of phosphate compounds
	Milk	Detection of effectiveness of pasteurization
Nucleases	Flavor enhancers	Production of nucleotides and nucleosides
Peroxidases (useful)	Vegetables	Detection of effectiveness of blanching
	Glucose determinations	In combination with glucose oxidase

Enzyme	*Food*	*Purpose or Action*
Peroxidases (deteriorative)	Vegetables	Off-flavors
	Fruits	Contribution to browning action
Catalase	Milk	Destruction of H_2O_2 in cold pasteurization
	Variety of products	To remove glucose and/or oxygen to prevent browning and/or oxidation; used in conjunction with glucose oxidase
Glucose oxidase	Variety of products	Removal of oxygen and/or glucose from products such as beer, cheese, carbonated beverages, dried eggs, fruit juices, meat and fish, milk powder, wine to prevent oxidation and/or browning; used in conjunction with catalase
	Glucose determination	Specific determination of glucose; used in conjunction with peroxidase
Polyphenol oxidase (useful)	Tea, coffee, tobacco	Development of browning during ripening, fermentation, and/or aging process
Polyphenol oxidase (deteriorative)	Fruits, vegetables	Browning, off-flavor development, loss of vitamins
Lipoxygenase	Vegetables	Destruction of essential fatty acids and vitamin A; development of off-flavors
Ascorbic acid oxidase	Vegetables, fruits	Destruction of vitamin C (ascorbic acid)
Thiaminase	Meats, fish	Destruction of thiamine

11.4 Summary

1 What is the nature and functions of enzymes?
2 What are the types of enzymes?
3 What are the functions of proteases?
4 Explain the functions of oxidoreductases?
5 List out various enzymes present in foods?
6 Discuss about the purpose of the enzymes?

11.6 Reference Books:

1 H.D.Belitz, W.Grosch and P.Schieber. Food Chemistry 4th revised and extended edition 2009
2 John M. deMan, Principles of food chemistry, Third edition, 1999
3 Upadhyay and Upadhyay, Biophysical chemistry, 2006
4 Albert L. Lehninger and David L.Nelson Principles of Biochemistry, 5th edition
5 Lillian Hoagland Meyer, Food chemistry, Litton Educational Publishing inc, U.S.A.

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Lesson – 12

ASH AS AN INDICATOR OF TOTAL MINERAL CONTENT

12.0 Objcective

After going through this lesson you will learn:

- Importance of ash in food analysis
- Various methods used in analysis of ash

Structure

12.1 Introduction

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff. A basic knowledge of the characteristics of various ashing procedures and types of equipment is essential to ensure reliable results. Two major types of ashing are used: dry ashing, primarily for proximate composition and for some types of speciûc mineral analyses; wet ashing (oxidation), as a preparation for the analysis of certain minerals. Microwave systems now are available for both dry and wet ashing, to speed the processes. Most dry samples (i.e., whole grain, cereals, and dried vegetables) need no preparation, while fresh vegetables need to be dried prior to ashing. High-fat products such as meats may need to be dried and fat extracted before ashing. The ash content of foods can be expressed on either a wet weight (as is) or on a dry weight basis.

12.2 Importance of Ash in Food Analysis

Ash content represents the total mineral content in foods. Determining the ash content may be important for several reasons. It is a part of proximate analysis for nutritional evaluation. Ashing is the ûrst step in preparing a food sample for speciûc elemental analysis. Because certain foods are high in particular minerals, ash content becomes important. One can usually expect a constant elemental content from the ash of animal products, but that from plant sources is variable.

Ash Content of Selected Foods

Food item	Percent Ash (wet weight basis)
Cereals, bread and pasta, rice brown, long-grain raw	1.5
Corn meal, whole grain	1.1
White rice, long grain, yellow	0.9
Wheat flour, whole grain	1.6
Rye bread	2.5
Milk, reduced fat, fluid, 2 %	0.7
Evaporated milk, canned, with added vitamin A	1.6
Butter, with salt	2.1
Yougurt, plain, low fat	1.1
Fruits and vegetablesApples, raw, with skin	0.2
Tomatoes, red, ripe, raw	0.5
Eggs, whole,raw, fresh	0.9
Chicken, broilers or fryers, breast meat only, raw	1.0

From US Department of Agriculture, Agricultural Research Service (2009) USDA National Nutrient Database for Standard Reference.

12.3 Methods

12.3.1 Sample Preparation

It cannot be overemphasized that the small sample used for ash, or other determinations, needs to be very carefully chosen so that it represents the original materials. A 2–10g sample generally is used for ash determination. For that purpose, milling, grinding, and the like probably will not alter the ash content much; however, if this ash is a preparatory step for speciûc mineral analyses, contamination by microelements is of potential concern. Remember, most grinders and

mincers are of steel construction. Repeated use of glassware can be a source of contaminants as well. The water source used in dilutions also may contain contaminants of some microelements. Distilled deionized water always should be used.

Plant Materials

Plant materials are generally dried by routine methods prior to grinding. The temperature of drying is of little consequence for ashing. However, the sample may be used for multiple determinations – protein, fiber, and so on – which require consideration of temperature for drying. Fresh stem and leaf tissue probably should be dried in two stages (i.e., first at a lower temperature of 550^{0}C, then a higher temperature) especially to prevent artifact lignin. Plant material with 15% or less moisture may be ashed without prior drying.

Fat and Sugar Products

Animal products, syrups, and spices require treatments prior to ashing because of high fat, moisture (spattering, swelling), or high sugar content (foaming) that may result in loss of sample. Meats, sugars, and syrups need to be evaporated to dryness on a steam bath or with an infrared (IR) lamp. One or two drops of olive oil (which contains no ash) are added to allow steam to escape as a crust is formed on the product. Smoking and burning may occur upon ashing for some products (e.g., cheese, seafood, spices). Allow this smoking and burning to finish slowly by keeping the muffle door open prior to the normal procedure. A sample may be ashed after drying and fat extraction. In most cases, mineral loss is minimal during drying and fat extraction. Under no circumstances should fat-extracted samples be heated until all the ether has been evaporated.

12.3.2 Dry Ashing

Dry ashing is incineration at high temperature (525^{0}C or higher). Incineration is accomplished with a muffle furnace. Several models of muffle furnaces are available, ranging from large-capacity units requiring either 208 or 240V supplies to small bench top units utilizing 110V outlets. Crucible selection becomes critical in ashing because the type depends upon the specific use.

Quartz crucibles are resistant to acids and halogens, but not alkali, at high temperatures. Vycor brand crucibles are stable to 900^{0}C, but Pyrex R R Gooch crucibles are limited to 500^{0}C. Ashing at a lower temperature of 500–525^{0}C may result in slightly higher ash values because of less decomposition of carbonates and loss of volatile salts. Porcelain crucibles resemble quartz crucibles in their properties, but will crack with rapid temperature changes. Porcelain crucibles are relatively inexpensive and usually the crucible of choice. Steel crucibles are resistant to both acids and alkalies and are inexpensive, but they are composed of chromium and nickel, which are possible sources of contamination. Platinum crucibles are very inert and are probably the best crucibles, but they are currently far too expensive for routine use for large numbers of samples. Quartz ûber crucibles are disposable, unbreakable, and can withstand temperatures up to 1000^{0}C. They are porous, allowing air to circulate around the sample and speed combustion. This reduces ashing times significantly and makes them ideal for solids and viscous liquids. Quartz ûber also cools in seconds, virtually eliminating the risk of burns.

All crucibles should be marked for identiûcation. Marks on crucibles with a felt-tip marking pen will disappear during ashing in a muffle furnace. Laboratory inks scribed with a steel pin are available commercially. Crucibles also may be etched with a diamond point and marked with a

0.5*M* solution of FeCl, in 20% HCl. An iron nail dissolved in concentrated HCl forms brown goo that is a satisfactory marker.

The crucibles should be ûred and cleaned prior to use. The advantages of conventional dry ashing are that it is a safe method, it requires no added reagents or blank subtraction, and little attention is needed once ignition begins. Usually a large number of crucibles can be handled at once, and the resultant ash can be used additionally in other analyses for most individual elements, acid-insoluble ash, and water-soluble and insoluble ash. The disadvantages are the length of time required (12–18h or overnight) and expensive equipment. There will be a loss of the volatile elements and interactions between mineral components and crucibles. Volatile elements at risk of being lost include As, B, Cd, Cr, Cu, Fe, Pb, Hg, Ni, P, V, and Zn.

12.3.3 Wet Ashing

Wet ashing is sometimes called wet oxidation or wet digestion. Its primary use is preparation for speciûc mineral analysis and metallic poisons. Often, analytical testing laboratories use only wet ashing in preparing samples for certain mineral analyses (e.g., Fe, Cu, Zn, P), because losses would occur by volatilization during dry ashing. There are several advantages to using the wet ashing procedure. Minerals will usually stay in solution, and there is little or no loss from volatilization because of the lower temperature. The oxidation time is short and requires a hood, hot plate, and long tongs, plus safety equipment. The disadvantages of wet ashing are that it takes virtually constant operator attention, corrosive reagents are necessary, and only small numbers of samples can be handled at any one time. If the wet digestion utilizes perchloric acid, all work needs to be carried out in an expensive special fume hood called a perchloric acid hood. Unfortunately, a single acid used in wet ashing does not give complete and rapid oxidation of organic material, so a mixture of acids often is used. Combinations of the following acid solutions are used most often:

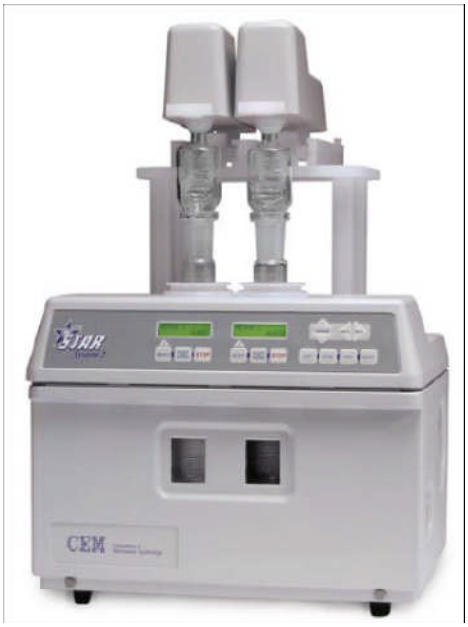
- nitric acid,
- sulfuric acid-hydrogen peroxide, and
- perchloric acid.

Different combinations are recommended for different types of samples. The nitric–perchloric combination is generally faster than the sulfuric–nitric procedure. While wet digestion with perchloric acid is an AOAC procedure (e.g., AOAC Method 975.03), many analytical laboratories avoid if possible the use of perchloric acid in wet ashing and instead use a combination of nitric acid with either sulfuric acid, hydrogen peroxide, or hydrochloric acid. Wet oxidation with perchloric acid is extremely dangerous since the perchloric acid has a tendency to explode. The perchloric acid hood that must be used has wash-down capabilities and does not contain plastic or glycerol-base caulking compounds. Precautions for use of perchloric acid are found in the AOAC methods under “Safe Handling of Special Chemical Hazards.” Cautions must be taken when fatty foods are wet ashed using perchloric acid. While perchloric acid does not interfere with atomic absorption spectroscopy, it does interfere in the traditional colorimetric assay for iron by reacting with iron in the sample to form ferrous perchlorate, which forms an insoluble complex with the *o*-phenanthrolene in the procedure.

12.3.4 Microwave Ashing

Both wet ashing and dry ashing can be done using microwave instrumentation, rather than the conventional dry ashing in a muffle furnace and wet ashing in a muffle or beaker on a hot plate.

The CEM Corporation (Matthews, NC) has developed a series of instruments for dry and wet ashing, as well as other laboratory systems for microwave-assisted chemistry. While the ashing procedures by conventional means can take many hours, the use of microwave instrumentation can reduce sample preparation time to minutes, allowing laboratories to increase their sample throughput significantly. This advantage has led to widespread use of microwave ashing, especially for wet ashing, both within analytical laboratories and quality control laboratories within food companies.



Microwave open vessel system

12.3.4.1 Microwave Wet Ashing

Microwave wet ashing (acid digestion) may be performed safely in either an open or closed-vessel microwave system. Choice of the system depends on the amount of sample and the temperatures required for digesting. Because of the ability of the closed vessels to contain higher pressures (some vessels can handle up to 1500 psi), acids may be heated past their boiling points. This ensures a more complete dissolution of hard-to-digest substances. It also allows the chemist to use nitric acid with samples that might normally require a harsher acid, such as sulfuric or perchloric. In closed vessels speciûcally designed for high- temperatures/high-pressure reactions, nitric acid can reach a temperature of 240æ%C. Thus, nitric acid is often the acid of choice, though hydrochloric, hydroûuoric, and sulfuric acids also are used, depending on the sample and the subsequent analysis being performed. Closed-vessel microwave digestion systems can process up to 40 samples at a time, with vessel liners available in Teûon R TM, TFM Fluoropolymer, and quartz. These systems allow the input of time, temperature, and pressure parameters in a step-by- step format (ramping). In addition, some instruments enable the user to adjust the power and offer “change- on-the-fly” software, which allows the method to be changed while the reaction is running.

Typically, in a closed-vessel microwave system, sample is placed in vessels with the appropriate amount of acid. The vessels are sealed and set on a carousel where the temperature and pressure sensors are connected to a control vessel. The carousel then is placed in the

microwave cavity, and the sensors are connected to the instrument. Time, temperature, pressure, and power parameters are chosen and the unit is started. Digestions normally take less than 30min. Because of the pressure generated by raising the temperature of a reaction, the vessels must be allowed to cool before being opened. The ability to process multiple samples simultaneously provides the chemist with greater throughput than traditional methods. (Note that some closed-vessel microwave digestion systems may also be used for acid concentration, solvent extraction, protein hydrolysis, and synthesis with the proper accessories.)

Open-vessel digestion systems are used often for larger sample sizes (up to 10g) and for samples that generate substantial amounts of gas as they are digested. Open-vessel systems can process up to six samples, each according to its own parameters in a sequential or simultaneous format. Teflon, quartz, or Pyrex R R vessels are used, and condensers are added for refluxing. Acid (reagent) is automatically added according to the programmed parameters. Sulfuric and nitric acids are used most often with open-vessel systems, as they process reactions under atmospheric conditions; however, hydrochloric and hydrofluoric acids, as well as hydrogen peroxide, can be used.

Microwave closed vessel digestion system

These instruments do not require the use of a fume hood, because a vapor containment system contains and neutralizes harmful fumes. Generally, in an open-vessel microwave system, the sample is placed in a vessel and the vessel is set in a slot in the microwave system. Time, temperature, and reagent addition parameters are then chosen. The unit is started, the acid is added, and the vapor containment system neutralizes the fumes from the reaction. Samples are typically processed much faster and more reproducibly than on a conventional hot plate. (Note that some open-vessel systems may be used for evaporation and acid concentration as well.)

12.3.4.2 Microwave Dry Ashing

Compared with conventional dry ashing in a mufûe furnace that often takes many hours, microwave mufûe furnaces can ash samples in minutes, decreasing analysis time by as much as 97%. Microwave mufûe furnaces can reach temperatures of up to 1200^0C. These systems may be programmed with various methods and to automatically warm up and cool down. In addition,

they are equipped with exhaust systems that circulate the air in the cavity to help decrease ashing times. Some also have scrubber systems to neutralize any fumes. Any crucible that may be used in a conventional muffle furnace may be used in a microwave furnace, including those made of porcelain, platinum, quartz, and quartz fiber. Quartz fiber crucibles cool in seconds and are not breakable. Some systems can process up to 15 (25ml) crucibles at a time. Typically, in microwave dry ashing, a desiccated crucible is weighed and then sample is added and it is weighed again. The crucible then is placed in the microwave furnace, and the time and temperature parameters are set.

Microwave dry ashing

A step-by-step (ramping) format may be used when programming the method. The system is started and the program is run to completion. The crucible then is carefully removed with tongs and reweighed. The sample then may be further analyzed, if necessary. Some tests call for acid to be added to a dry ashed sample, which is then digested for further analysis. A comparative study showed that dry ashing various plants for 40min using a microwave system (CEM Corporation, Matthews NC) was similar to the 4-h time in a conventional muffle furnace. Twenty minutes was shown to be adequate for the plant material used except for Cu determinations, which needed 40min to obtain similar results. Other comparative examples include dried egg yolks, which can be ashed in 20 min in a microwave system, but require 4h in a conventional muffle furnace. It takes 16h to ash lactose in a conventional muffle furnace, but only 35min in a microwave furnace. Though microwave furnaces may not hold as many samples as a conventional furnace, their speed actually allows significantly more samples to be processed in the same amount of time. Also, microwave furnaces do not require fume hood space.

12.4 Summary

Ash refers to the inorganic residue remaining after either ignition or complete oxidation of organic matter in a foodstuff. A basic knowledge of the characteristics of various ashing procedures and types of equipment is essential to ensure reliable results. Two major types of ashing are used: dry ashing, primarily for proximate composition and for some types of speciûc mineral analyses; wet ashing (oxidation), as a preparation for the analysis of certain minerals. the small sample used for ash, or other determinations, needs to be very carefully chosen so that it represents the original materials. A 2–10g sample generally is used for ash determination. For that purpose, milling, grinding, and the like probably will not alter the ash content much; however, if this ash is a preparatory step for specific mineral analyses, contamination by microelements is of potential concern.

The two major types of ashing, dry ashing and wet oxidation (ashing), can be done by conventional means or using microwave systems. The procedure of choice depends upon the use of ash following its determination, and limitations based on cost, time, and sample numbers. Conventional dry ashing is based upon incineration at high temperatures in a muffle furnace. Except for certain elements, the residue may be used for further specific mineral analyses. Wet ashing (oxidation) often is used as a preparation for specific elemental analysis by simultaneously dissolving minerals and oxidizing all organic material. Wet ashing conserves volatile element, but requires more operator time than dry ashing and is limited to a smaller number of samples. Dry and wet ashing using microwave technology reduces the time for analyses and requires little additional equipment (special fume hood) or space (heat room).

12.5 Self Assessment Questions

1. Write an account on importance of ash in food analysis

2. How the sample is prepared for ash analysis?
3. Write notes on
   a. Dry ashing
   b. Wet ashing
4. Explain in detail about
   a. Microwave wet ashing
   b. Microwave dry ashing

12.6 Reference Books

1. S. Suzanne Nielsen, Food analysis, 4th edition, 2010
2. Upadhyay and Upadhyay, Biophysical chemistry, 2006
3. Clifton.E.Meloan, food analysis 3rd edition (theory and practice)

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Lesson - 13

CHROMATOGRAPHY- I

13.0 Objective

After going through this lesson you will learn

- The concept of adsorption chromatography
- The concept of column liquid chromatography

Structure

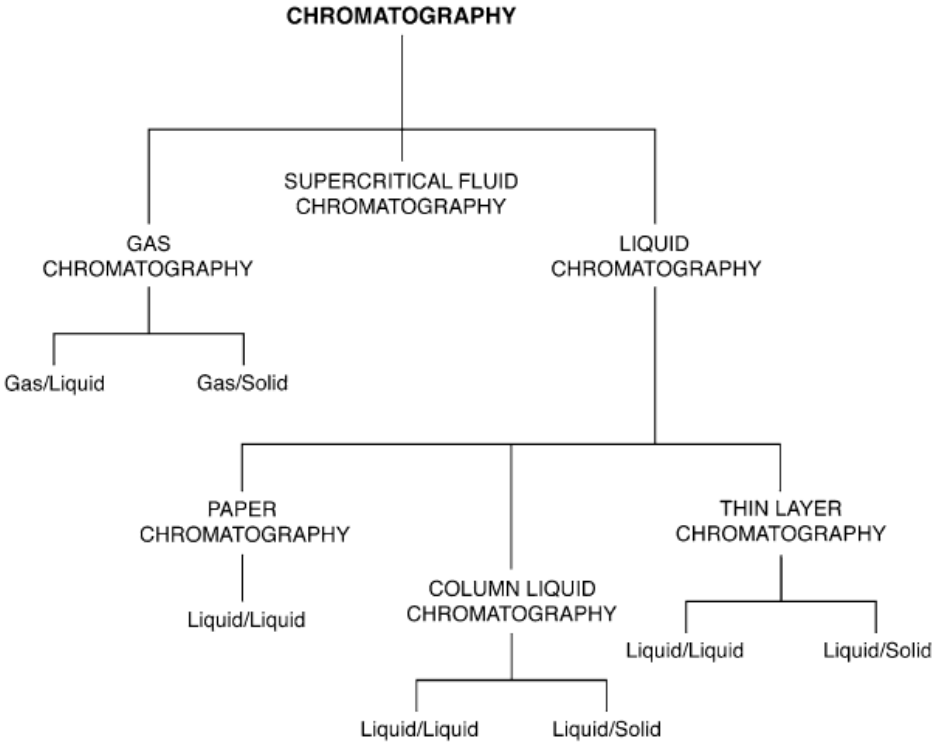
13.1 Introduction

Chromatography has a great impact on all areas of analysis and, therefore, on the progress of science in general. Chromatography differs from other methods of separation in that a wide variety of materials, equipment, and techniques can be used. Chromatography is a general term applied to a wide variety of separation techniques based on the partitioning or distribution of a sample (solute) between a moving or mobile phase and a fixed or stationary phase.

In its simplest form, extraction refers to the transfer of a solute from one liquid phase to another. Extraction in myriad forms is integral to food analysis – whether used for preliminary sample cleanup, concentration of the component of interest, or as the actual means of analysis. Extractions may be categorized as batch, continuous, or countercurrent processes.

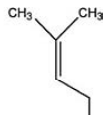
Chromatography may be viewed as a series of equilibrations between the mobile and stationary phase. The relative interaction of a solute with these two phases is described by the partition (*K*) or distribution (*D*) coefficient (ratio of concentration of solute in stationary phase to concentration of solute in mobile phase). The mobile phase may be either a gas (GC) or liquid (LC) or a supercritical fluid (SFC). The stationary phase may be a liquid or, more usually, a solid. The

field of chromatography can be subdivided according to the various techniques applied or according to the physicochemical principles involved in the separation. In as much as the nature of interactions between solute molecules and the mobile or stationary phases differ, these methods have the ability to separate different kinds of molecules.



Scheme for subdividing the field of chromatography, according to various applied techniques

Method	Mobile/stationary phase	Retension varies with
Gas-liquid chromatography	Gas/liquid	Molecular size/polarity
Gas-solid chromatography	Gas/solid	Molecular size/polarity
Supercritical fluid chromatography	Supercritical fluid/solid	Molecular size/polarity
Reversed-phase chromatography	Polar liquid/nonpolar liquid or solid	Molecular size/polarity
Normal-phase chromatography	Less polar liquid/more polar liquid or solid	Molecular size/polarity
Ion-exchange chromatography	Less polar liquid/more polar liquid or solid	Molecular size/polarity
Size-exclusion chromatography	Liquid/solid	Molecular size



Hydrophobic-interaction Chromatography	Polar liquid/nonpolar liquid or solid	Molecular size/polarity
Affinity chromatography	Water/binding sites	Specific structure

Characteristics of different chromatographic methods

13.2 Adsorption (Liquid–Solid) Chromatography

In this chromatographic mode, the stationary phase is a finely divided solid to maximize the surface area. The stationary phase (adsorbent) is chosen to permit differential interaction with the components of the sample to be resolved. The intermolecular forces thought to be primarily responsible for chromatographic adsorption include the following:

- Vander Waals forces
- Electrostatic forces
- Hydrogen bonds
- Hydrophobic interactions

Sites available for interaction with any given substance are heterogeneous. Binding sites with greater affinities, the most active sites, tend to be populated first, so that additional solutes are less firmly bound. The net result is that adsorption is a concentration-dependent process, and the adsorption coefficient is not a constant. Sample loads exceeding the adsorptive capacity of the stationary phase will result in relatively poor separation.

Classic adsorption chromatography utilizes silica (slightly acidic), alumina (slightly basic), charcoal (nonpolar), or a few other materials as the stationary phase. Both silica and alumina possess surface hydroxyl groups, and Lewis acid-type interactions determine their adsorption characteristics. The elution order of compounds from these adsorptive stationary phases can often be predicted on the basis of their relative polarities. Compounds with the most polar functional groups are retained most strongly on polar adsorbents and, therefore, are eluted last. Nonpolar solutes are eluted first. One model proposed to explain the mechanism of liquid–solid chromatography is that solute and solvent molecules are competing for active sites on the adsorbent. Thus, as relative adsorption of the mobile phase increases, adsorption of the solute must decrease.

Solvents can be rated in order of their strength of adsorption on a particular adsorbent, such as silica. Such a solvent strength (or polarity) scale is called a eluotropic series. Silica has a similar rank ordering. Once an adsorbent has been chosen, solvents can be selected from the eluotropic series for that adsorbent. Mobile phase polarity can be increased (often by admixture of more polar solvents) until elution of the compound(s) of interest has been achieved. Adsorption chromatography separates aromatic or aliphatic nonpolar compounds, based primarily on the type and number of functional groups present.

The labile, fat-soluble chlorophyll and carotenoid pigments from plants have been studied extensively by adsorption column chromatography. Adsorption chromatography also has been used for the analysis of fat-soluble vitamins. Frequently, it is used as a batch procedure for removal of impurities from samples prior to other analyses. For example, disposable solid phase extraction cartridges containing silica have been used for food analyses, such as lipids in soybean oil, carotenoids in citrus fruit, and vitamin E in grain.

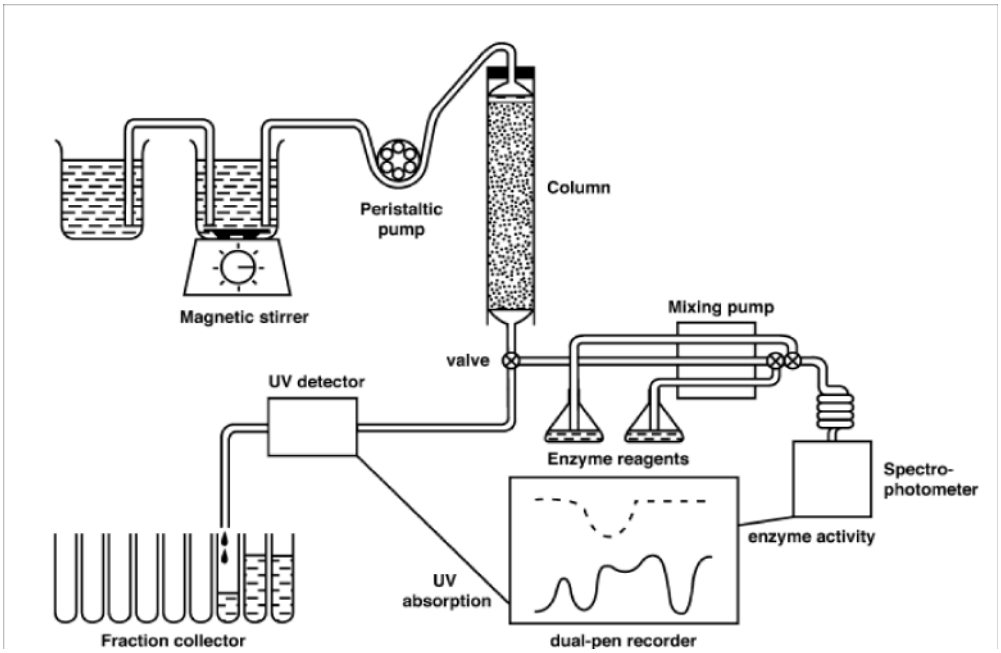
13.2.1 Applications

Adsorption chromatography has been extensively used for biochemical separations. The long list includes amino acids, mono and disaccharides, neutral lipids, cholesterol esters, carotenoids, phospholipids, etc.

13.3 Column Liquid Chromatography

Column chromatography is the most useful method of separating compounds in a mixture. Fractionation of solutes occurs as a result of differential migration through a closed tube of stationary phase, and analytes can be monitored while the separation is in progress. In column liquid chromatography, the mobile phase is liquid and the stationary phase can be either solid or liquid supported by an inert solid. A system for low-pressure. Having selected a stationary and mobile phase suitable for the separation problem at hand, the analyst must first prepare the stationary phase (resin, gel, or packing material) for use according to the supplier's instructions. (For example, the stationary phase often must be hydrated or preswelled in the mobile phase). The prepared stationary phase then is packed into a column (usually glass), the length and diameter of which are determined by the amount of sample to be loaded, the separation mode to be used, and the degree of resolution required. Longer and narrower columns usually enhance resolution and separation.

Adsorption columns may be either dry or wet packed; other types of columns are wet packed. The most common technique for wet packing involves making a slurry of the adsorbent with the solvent and pouring this into the column. As the sorbent settles, excess solvent is drained off and additional slurry is added. This process is repeated until the desired bed height is obtained. If the packing solvent is different from the initial eluting solvent, the column must be thoroughly washed (equilibrated) with the starting mobile phase. The sample to be fractionated, dissolved in a minimum volume of mobile phase, is applied in a layer at the top (or head) of the column. Classical or low pressure chromatography utilizes only gravity flow or a peristaltic pump to maintain a flow of mobile phase (eluent or eluting solvent) through the column.



low-pressure column liquid chromatography

In the case of a gravity-fed system, eluent is simply siphoned from a reservoir into the column. The flow rate is governed by the hydrostatic pressure, measured as the distance between the level of liquid in the reservoir and the level of the column outlet. If eluent is fed to the column by a peristaltic pump, then the flow rate is determined by the pump speed and, thus, regulation of hydrostatic pressure is not necessary. The process of passing the mobile phase through the column is called elution, and the portion that emerges from the outlet end of the column is sometimes called the elute (or effluent).

Elution may be isocratic (constant mobile-phase composition) or a gradient (changing the mobile phase, e.g., increasing solvent strength or pH) during elution in order to enhance resolution and decrease analysis time. As elution proceeds, components of the sample are selectively retarded by the stationary phase based on the strength of interaction with the stationary phase, and thus they are eluted at different times.

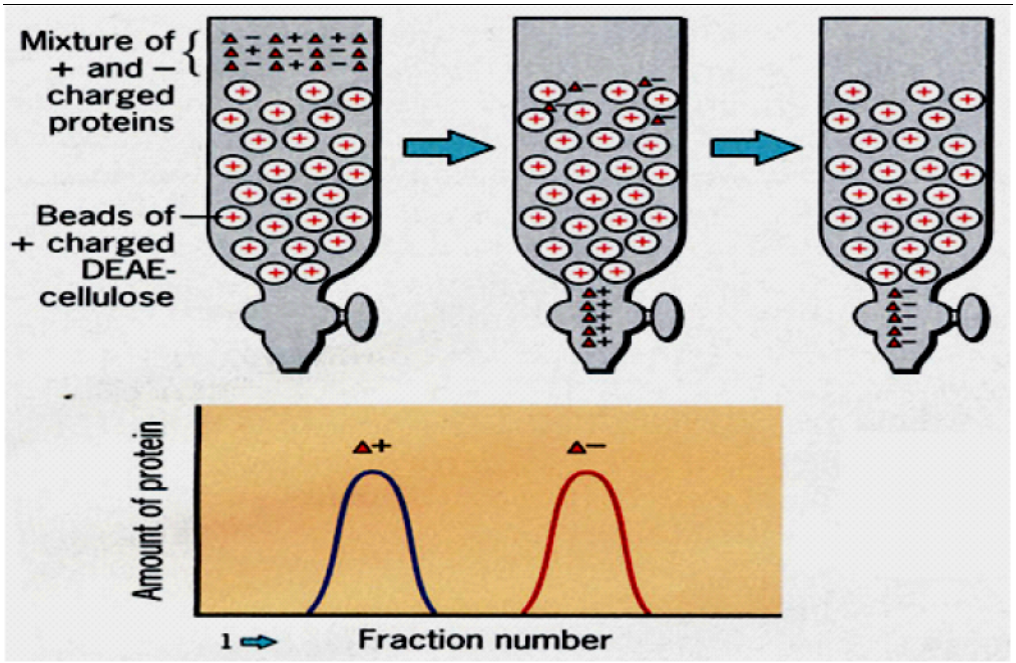
The column eluate may be directed through a detector and then into tubes, changed at intervals by a fraction collector. The detector response, in the form of an electrical signal, may be recorded (the chromatogram), using either a chart recorder or a computerized software, and used for qualitative or quantitative analysis, as discussed in more detail later. The fraction collector may be set to collect eluate at specified time intervals or after a certain volume or number of drops has been collected. Components of the sample that have been chromatographically separated and collected then can be further analyzed as needed.

13.3.1 Ion-Exchange Chromatography

Ion exchange is a separation/purification process occurring naturally, for example, in soils and is utilized in water softeners and deionizers. Three types of separation may be achieved:

- ionic from nonionic,
- cationic from anionic, and
- mixtures of similarly charged species.

In the first two cases, one substance binds to the ion-exchange medium, whereas the other substance does not. The stationary phase (ion exchanger) contains fixed functional groups that are either negatively or positively charged. Exchangeable counter ions preserve charge neutrality. A sample ion (or charged sites on large molecules) can exchange with the counter ion to become the partner of the fixed charge. The functional group of the stationary phase determines whether cations or anions are exchanged.



Ion exchange chromatography

Cation exchangers contain covalently bound negatively charged functional groups, whereas anion exchangers contain bound positively charged groups. The chemical nature of these acidic or basic residues determines how stationary-phase ionization is affected by the mobile-phase pH. The strongly acidic sulfonic acid moieties (RSO_3^-) of "strong"-cation exchangers are completely ionized at all pH values above 2. Strongly basic quaternary amine groups (RNR_3^+) on "strong"-anion exchangers are ionized at all pH values below 10.

Since maximum negative or positive charge is maintained over a broad pH range, the exchange or binding capacity of these stationary phases is essentially constant, regardless of mobile-phase pH. "Weak"-cation exchangers contain weakly acidic carboxylic acid functional groups, (RCO_2^-); consequently, their exchange capacity varies considerably between ca. pH 4 and 10. Weakly basic anion exchangers possess primary, secondary, or tertiary amine residues ($R\text{-}NHR_2^+$), which are deprotonated in moderately basic solution, thereby losing their positive charge and the ability to bind anions. Thus, one way of eluting solutes bound to an ion-exchange medium is to change the mobile-phase pH. A second way to elute bound solutes is to increase the ionic strength (e.g., use NaCl) of the mobile phase, to weaken the electrostatic interactions. Chromatographic separations by ion exchange are based upon differences in affinity of the exchangers for the ions (or charged species) to be separated.

The factors that govern selectivity of an exchanger for a particular ion include the ionic valence, radius, and concentration; the nature of the exchanger (including its displaceable counterion); and the composition and pH of the mobile phase. To be useful as an ion exchanger, a material must be both ionic in nature and highly permeable. Synthetic ion exchangers are thus cross-linked polyelectrolytes, and they may be inorganic (e.g., aluminosilicates) or, more commonly, organic compounds.

Polystyrene, made by crosslinking styrene with divinyl benzene (DVB), may be modified to produce either anion- or cation-exchange resins. Polymeric resins such as these are commercially available in a wide range of particle sizes and with different degrees of cross-linking (expressed as weight percent of DVB in the mixture). The extent of cross-linking controls the rigidity and porosity of the resin, which, in turn, determines its optimal use. Lightly cross-linked resins permit rapid equilibration of solute, but particles swell in water, thereby decreasing charge density and selectivity (relative affinity) of the resin for different ions. More highly cross-linked resins exhibit less swelling, higher exchange capacity, and selectivity, but longer equilibration times.

The small pore size, high charge density, and inherent hydrophobicity of the older ion-exchange resins have limited their use to small molecules (molecular weight (MW) <500). Ion exchangers based on polysaccharides, such as cellulose, dextran, or agarose, have proven very useful for the separation and purification of large molecules, such as proteins and nucleic acids. These materials, called gels, are much softer than polystyrene resins, and thus may be derivatized with strong or with weak acidic or basic groups via OH moieties on the polysaccharide backbone. They have much larger pore sizes and lower charge densities than the older synthetic resins.

Applications

The separation of

- Amino acids, sugars, alkaloids, and proteins
- Plant pigments, pesticides, drugs and their metabolites
- Animal and plant hormones and complex lipids

13.3.2 Gel filtration chromatography

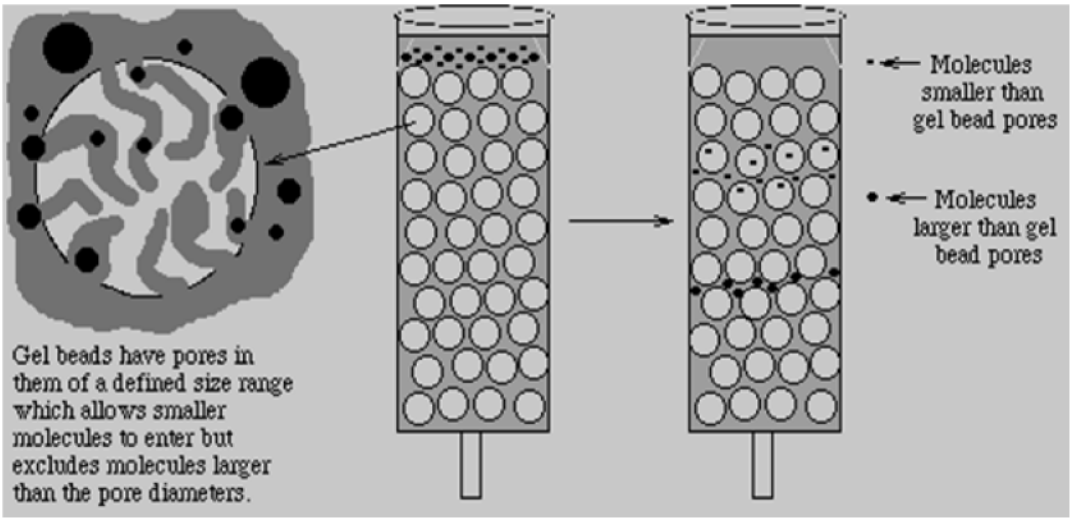
Gel filtration separates molecules according to differences in size as they pass through a gel filtration medium packed in a column. Unlike ion exchange or affinity chromatography, molecules do not bind to the chromatography medium so buffer composition does not directly affect resolution. Consequently, a significant advantage of gel filtration is that conditions can be varied to suit the type of the sample or the requirement for further purification, analysis or storage without altering the separation.

Gel filtration is well suited for biomolecules that may be sensitive to changes in pH, concentration of metal ions or co-factors and harsh environmental conditions. Separations can be performed in the presence of essential ions or cofactors, detergents, urea, guanidine hydrochloride, at high or low ionic strength, at 37^0c or in the cold room according the requirements of the experiment.

To perform a separation, gel filtration medium is packed into a column to form a packed bed. The medium is a porous matrix in the form of spherical particles that have been chosen for their chemical and physical stability, and inertness. The packed bed is equilibrated with buffer which fills the pores of the matrix and the space in between the particles. The liquid inside the pores is sometimes referred to as the stationary phase and this liquid is in equilibrium with the liquid outside the particles, referred to as the mobile phase. It should be noted that samples are eluted isocratically i.e. there is no need to use different buffers during the separation. However, a wash step using the running buffer is usually included at the end of a separation to facilitate the removal of any molecules that may have been retained on the column and to prepare the column for a new run.

This technique is used in fractionation mode to separate multiple components in a sample on the basis of differences in their size. The goal may be to isolate one or more of the components, to determine molecular weight, or to analyze the molecular weight distribution in the sample to determine molecular weight, or to analyze the molecular weight distribution in the sample. The best results for high resolution fractionation will be achieved with samples that originally contain few components or with samples that have been partially purified by other chromatography techniques.

Many factors influence the final resolution: sample volume, the ratio of sample volume to column volume, column dimensions, particle size, particle size distribution, packing density , pore size of the particles, flow rate, and viscosity of the sample and buffer. The molecular weight range over which a gel filtration medium can separate molecules is referred to as the selectivity of the medium.



Gel filtration chromatography

Applications

- Determination of molecular weight of macromolecules.
- Dilute solutions of macromolecules with molecular weights higher than the exclusion limit may be readily concentrated by utilizing the hygroscopic nature of the dry gel. Sephadex G-200 absorbs 20 times its weight of water, although G-25 is preferred for its rapid action. This treatment leaves the macromolecular solution concentrated but at the same time unaltered in pH or ionic strength.
- Removal of salts and small molecules from macromolecules can be easily performed using gel filtration since the distribution coefficients of salt molecules will be largely different from those of macromolecules.

- Proteins, enzymes, hormones, antibodies, nucleic acids, polysaccharides, and even viruses have been separated in various experiments which have used different types of gels or glass granules.

13.4 Summary

Chromatography is a separation method based on the partitioning of a solute between a mobile phase and a stationary phase. The mobile phase may be liquid, gas, or a supercritical fluid. The stationary phase may be an immobilized liquid or a solid, in either a planar or column form. Based on the physicochemical characteristics of the analyte, and the availability of instrumentation, a chromatographic system is chosen to separate, indentify, and quantify the analyte. Chromatographic modes include adsorption, partition, ion exchange, size exclusion, and affinity chromatography. Factors to be considered when developing a separation include mobile phase variables (strength, pH, temperature, and flow rate), and column efficiency, selectivity, and capacity. Following detection, a chromatogram provides both qualitative and quantitative information via retention time and peak height area data.

13.5 Self Assessment Questions

1 What are the applications of adsorption liquid chromatography?
2 Explain in detail about the column liquid chromatography?
3 How column liquid chromatography is used in analysis of various foods?
4 What is the principle of ion exchange chromatography?
5 Explain about principle and applications of gel filtration chromatography?

13.6 Reference Books

1. S. Suzanne Nielsen, Food analysis, 4th edition, 2010
2. Upadhyay and Upadhyay, Biophysical chemistry, 2006
3. Clifton.E.Meloan, food analysis 3rd edition (theory and practice)

Dr. P. KIRANMAYI

LESSON - 14

CHROMATOGRAPHY – II

14.0 Objective

After going through this lesson you will learn:

- The concept of Gas chromatography
- The concept of High performance liquid chromatography

Structure

14.1 Introduction

Chromatography has a great impact on all areas of analysis and, therefore, on the progress of science in general. Chromatography differs from other methods of separation in that a wide variety of materials, equipment, and techniques can be used. Chromatography is a general term applied to a wide variety of separation techniques based on the partitioning or distribution of a sample (solute) between a moving or mobile phase and a fixed or stationary phase.

Chromatography may be viewed as a series of equilibrations between the mobile and stationary phase. The relative interaction of a solute with these two phases is described by the partition (K) or distribution (D) coefficient (ratio of concentration of solute in stationary phase to concentration of solute in mobile phase). The mobile phase may be either a gas (GC) or liquid (LC) or a supercritical fluid (SFC). The stationary phase may be a liquid or, more usually, a solid. The field of chromatography can be subdivided according to the various techniques applied or according to the physicochemical principles involved in the separation. In as much as the nature of interactions

between solute molecules and the mobile or stationary phases differ, these methods have the ability to separate different kinds of molecules.

14.2 Gas Chromatography

GC has found broad application in both the food industry and academia. It is exceptionally well suited to the analysis of volatile thermally stable compounds. This is due to the outstanding resolving properties of the method and the wide variety of detectors that can provide either sensitivity or selectivity in analysis. Sample preparation generally involves the isolation of solutes from foods, which may be accomplished by headspace analysis, distillation, preparative chromatography (including solid-phase extraction), or extraction (liquid–liquid).

Some solutes can then be directly analyzed, while others must be derivatized prior to analysis. The gas chromatograph consists of a gas supply and regulators (pressure and flow control), injection port, column and column oven, detector, electronics, and a data recording and processing system. The analyst must be knowledgeable about each of these GC components: carrier and detector gases; injection port temperatures and operation in split, splitless, temperature-programmed, or on-column modes; column choices and optimization (gas flows and temperature profile during separation); and detectors (TCD, FID, NPD, ECD, FPD, PFPD, and PID). The characteristics of these GC components and an understanding of basic chromatographic theory are essential to balancing the properties of resolution, capacity, speed, and sensitivity.

Unlike most of the other chromatographic techniques, traditional GC has reached the theoretical limits in terms of both resolution and sensitivity. Thus, this method will not change significantly in the future other than for minor innovations in hardware or associated computer software. However, two-dimensional GCs, both heart-cut GC–GC and comprehensive GC × GC, are still developing quickly in both instrumentation and applications, especially in the field of flavor analysis. GC as a separation technique has been combined with AED, FTIR, and MS as detection techniques to make GC an even more powerful tool. Such hyphenated techniques are likely to continu

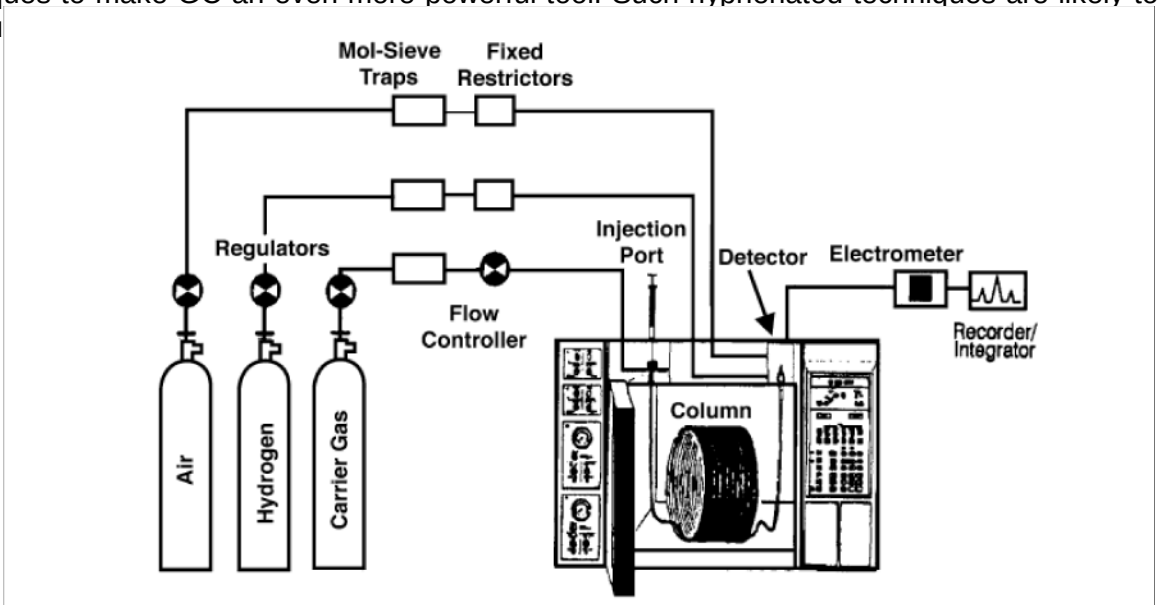


Diagram of a gas chromatographic system

14.2.1 Applications

1. Gas chromatography provides both qualitative and quantitative analysis of carbohydrates. For GC, sugars must be converted into volatile derivatives. The most commonly used derivatives are the alditol peracetates (and aldonic acid pertrimethylsilyl ethers from uronic acids). Conversion of sugars into peracetylated aldononitrile (aldoses) and peracetylated ketooxime (ketoses) derivatives for GC has also been done, although this procedure is not used nearly as much as the preparation of peracetylated aldoses and aldonic acids. A flame ionization detector is the detector of choice for peracetylated carbohydrate derivatives. The most serious problem with GC for carbohydrate analysis is that two preparation steps are involved: reduction of aldehyde groups to primary alcohol groups and conversion of the reduced sugar into a volatile peracetate ester or pertrimethylsilyl ether derivative. Of course, for the analysis to be successful, each of these steps must be 100% complete (i.e., stoichiometric).

2. Determination of the fatty acid composition of a product permits the calculation of the following categories of fats that pertain to health issues and food labeling: percent saturated fatty acids, percent unsaturated fatty acids, percent monounsaturated fatty acids, percent polyunsaturated fatty acids, CLAs, and percent *trans* isomer fatty acids. Calculation of fatty acids as a percentage is referred to as normalization, that is, the areas of all of the FAMEs are summed and the percent area of each fatty acid is calculated relative to the total area. This is a reasonable procedure because with flame ionization detectors (FID), the weight of fatty acids in a mixture closely parallels the area on the chromatogram. However, this is not absolutely correct. Theoretical correction factors are needed to correct for the FID response, which is different depending on the level of unsaturation in FAMEs. The separation of FAMEs on this SP2560 column is typical of what is seen when using a highly polar (biscyanopropyl polysiloxane) column.

14.3 Paper Chromatography

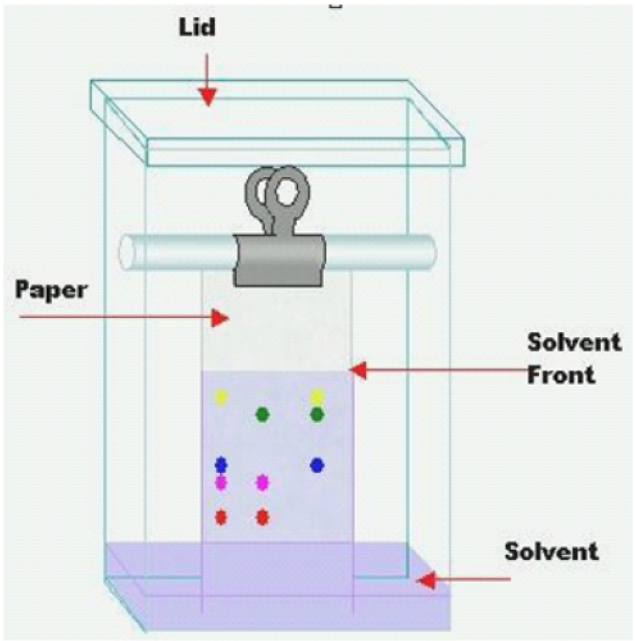
The paper commonly used consists of highly purified cellulose. Cellulose, a homopolysacchride of glucose, contains several thousand anhydro glucose units linked through oxygen atoms. Many of the hydroxyl groups of glucose, however, become partially oxidized during manufacture. The oxidation products usually are aldehyde, ketone or carboxyl functional groups. The paper also contains impurities through inorganic substances, adsorbed salts and mineral matter, which gets deposited on the paper while it is being processed. These impurities may be removed by washing the paper with 0.1 NHCl and drying it before chromatography is carried out. This gives better results. Cellulose fibers in the paper hold moisture tightly through formation of hydrogen bonds. The cellulose itself takes a negative charge in company of water. The paper exhibits weak ion exchange and adsorptive properties. Modified forms of paper have been produced in which the paper has been impregnated with alumina, silica gel, ion exchange resin, etc. while these modifications lead to different mechanisms of separation, the technique remains the same.

The apparatus required for paper chromatography consists of a support for a paper, a solvent trough, and an air tight chamber in which the chromatogram is developed. The sample is applied to the paper as a small spot. This is done before dipping the paper in to the eluting sample.

Any device, which can transfer a small volume of paper, can be used for spotting. Generally used devices are platinum loop, capillary tube, or a micropipette, of these, platinum is preferred because it can be reused with several substances after heating on a flame. A micropipette can also be reused after it tip has been disposed and a new tip applied. For some methods the sample may be applied as a narrow streak at right angles to the flow of solvent.

There are two main techniques, which may be employed for the development of paper chromatograms- ascending or descending techniques. In both cases the solvent is placed in the base of a sealed tank or glass jar to allow the chamber to become saturated with the solvent vapour. After equilibration of the chamber is achieved, the development of the chromatogram may be started. If the development is to be performed by the ascending technique, the paper is allowed to hand in or is suspended manner that the base of the paper is in contact with the solvent at the base of the chamber. The sample spots should be in a position just above the surface of the solvent so that as the solvent moves vertically up the paper by capillary action, separation of the sample is achieved. In the descending technique, the end of the paper near which the samples are located is held in a trough at the top of the tank and the rest of the paper to hang vertically but not in contact with the solvent in the base of the tank. Development is started by adding the solvent to the trough. Separation of the sample is achieved as the solvent moves downward under gravity.

Ascending has two advantages. Firstly, the setup required for it is very simple. Secondly, the resolution of sample by ascending technique is somewhat better as compared to the descending technique. This is so because in ascending chromatography, two forces are acting on the solute: the capillary force, which makes it move up, and the gravitational force which opposes this movement. Under the influence of these two forces the sample components are resolved better than in descending technique. The disadvantage of the ascending technique, however, is that it is very slow. The descending technique, on the other hand is much faster than the ascending technique. Based on the above advantages, one can choose the technique which suits one's purpose most.



Paper chromatography

A third, less used technique is the technique of radial development. In this method the sample is spotted at the center of a circularly cut disc of paper which is placed horizontally. The center of the paper is connected with a wick to the solvent, which is placed at the base of the jar. The solvent rises up the wick and thence in to the paper through capillary action. The sample components now move out ward radially forming concentric circles of increasing diameter. If the components to be separated are coloured, the chromatogram developed developed by this method looks pleasing to the eye. The resolution of the component by this technique is sharper. The apparatus is also simple. One way is to press the paper between the two glass plates with a whole in the center through which the wick can be connected for solvent supply. Alternatively, a large circular glass jar covered with a glass plate serves as a very good chamber.

A plane surface is amenable to sequential development in two directions using two different solvents. The paper, with the sample applied as a spot close to corner, is developed in the normal fashion by either ascending or descending procedure. The development is continued until the faster moving component or solvent front approaches the end of the paper. The paper is then removed and the solvent is allowed to evaporate. This paper is then turned 90^0 and developed a second time with another solvent having totally different eluting properties. Since the two solvents used have different properties, the distribution coefficients of individual components in them will also differ.

There are various methods of detection available. If the sample components are coloured. The analysis becomes simple as the distinctive colour itself identifies the component. When the components are colourless they can be imparted colour by spraying the paper with colour producing reagents. A case in point is the detection of amino acids. Ninhydrin reagent spread on the paper reacts with amines and amino acids to form a blue or purple colour.

The spots due to amino acids can now be detected on account of the blue colour that they develop. If not sprayed, the reagents may be applied to the paper by either immersing the paper in the reagent or by treating the paper to the reagent vapours. Other methods of detection are

- Ultraviolet and infrared absorption
- Fluorescence,
- Radioactivity

Otherwise, the components may be extracted and chemical an physical tests be performed on the extract. The identification of a given compound may be made on the basis of the distance traversed by solute relative to the distance moved by the solvent front. This ratio, which reflex the distribution coefficient of the given solute, is known as the retardation factor $R_{f,}$ and is constant for a given compound under standard conditions

$$R_f = \frac{\text{Distance moved by component}}{\text{Distance moved by solvent}}$$

14.3.1 Applications

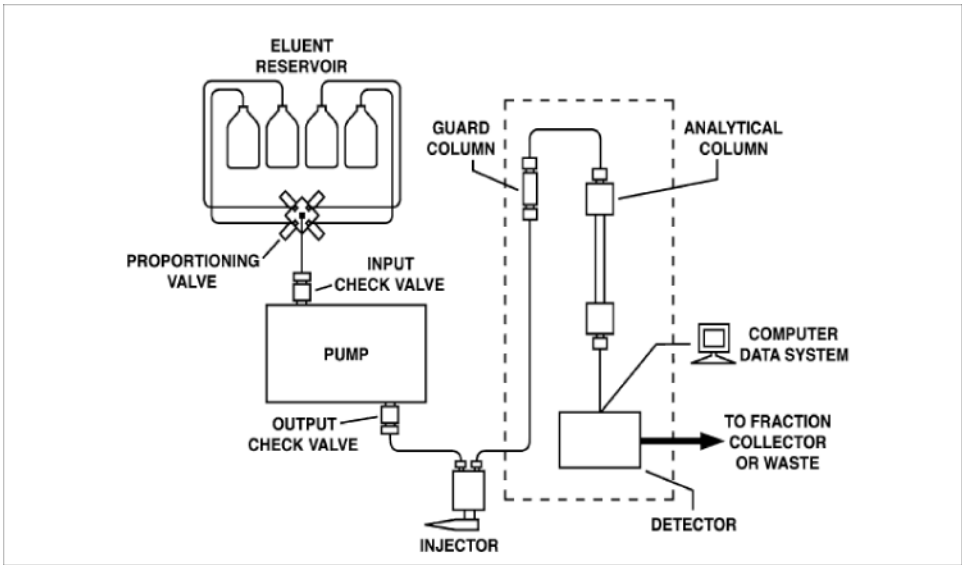
- The control of purity of pharmaceuticals
- The detection of adulterants and contaminants in foods and drinks
- The study of ripening and fermentation
- The detection of drugs and dopes in animals and humans

- The analyses of cosmetics and to top it all,
- The analyses of the reaction mixtures in biochemical labs are all performed routinely with paper chromatography technique.

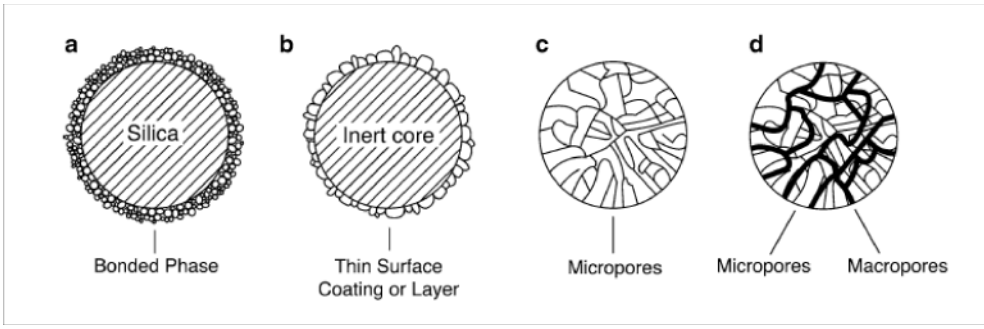
14.4 High-Performance Liquid Chromatography

HPLC is a chromatographic technique of great versatility and analytical power. A basic HPLC system consists of a pump, injector, column, detector, and data system. The pump delivers mobile phase through the system. An injector allows sample to be placed into the flowing mobile phase for introduction onto the column. The HPLC column consists of stainless steel or polymer hardware filled with a separation packing material. Various auxiliary columns, particularly guard columns, may be used prior to the analytical column. Detectors used in HPLC include UV-Vis absorption, fluorescence, RI, electrochemical, and light scattering, as well as coupled analytical systems, such as a mass spectrometer. Detection sensitivity or specificity sometimes can be enhanced by chemical derivatization of the analyte. Computer-controlled data station systems offer data collection and processing capabilities, and can run the instrument when an automated system is needed.

A broad variety of column packing materials have contributed greatly to the wide spread use of HPLC. These column packing materials may be categorized as silica-based (porous silica, bonded phases, pellicular packings) or polymeric (microporous, macroporous, or pellicular/ nonporous). The success of silica-based bonded phases has expanded the applications of normal-phase and reversed-phase modes of separation in HPLC. Separations also are achieved with ion-exchange, size-exclusion, and affinity chromatography. HPLC is widely used for the analysis of small molecules and ions, such as sugars, vitamins, and amino acids, and is applied to the separation and purification of macromolecules, such as proteins and polysaccharides.



Schematic representation of a system for high-performance liquid chromatography



Some types of packing materials utilized in HPLC

14.4.1 Normal-Phase HPLC

In the past, normal-phase HPLC was used for the analysis of fat-soluble vitamins, although reverse phase is currently applied more frequently for these analyses. Normal phase is currently used for the analyses of biologically active polyphenols from natural plant sources, such as grape and cocoa. It is also used for the analysis of relatively polar vitamins, such as vitamins A, D, E, and K, and also natural carotenoid pigments, which impart both color and health benefits to foods. Highly hydrophilic species, such as carbohydrates, also may be resolved by normal-phase chromatography, using amino bonded phase HPLC columns.

14.4.2 Reversed-Phase HPLC

Reversed-phase has been the HPLC mode most used for analysis of plant proteins. Cereal proteins, among the most difficult of these proteins to isolate and characterize, are now routinely analyzed by this method. Both water- and fat-soluble vitamins can be analyzed by reversed-phase HPLC, and the availability of fluorescence detectors has enabled researchers to quantitate very small amounts of the different forms of vitamin B6 (vitamers) in foods and biological samples. The separation of several of these vitamers in a rice bran extract achieved by reversed-phase ion-pair HPLC.

Reversed-phase ion-pair HPLC can be used to resolve carbohydrates on C18 bonded-phase columns, and the constituents of soft drinks (caffeine, aspartame, etc.) can be rapidly separated. Reversedphase HPLC using a variety of detection methods, including RI, UV, and light scattering, has been applied to the analysis of lipids. Antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), can be extracted from dry foods and analyzed with simultaneous UV and fluorescence detection. Phenolic flavor compounds (such as vanillin) and pigments (such as chlorophylls, carotenoids, and anthocyanins) are also easily analyzed. Reversed-phase ion-pair chromatography also is used for the separation of synthetic food colors.

14.4.3 Applications

HPLC has been successfully applied to the separation of:

- proteins, nucleic acids, polysaccharides
- plant pigments, amino acids, pesticides
- steroids, drugs and their metabolites, animal and plant hormones and complex lipids

14.5 Summary

The paper commonly used consists of highly purified cellulose. Cellulose, a homopolysacchride of glucose, contains several thousand anhydro glucose units linked through oxygen atoms. Many of the hydroxyl groups of glucose, however, become partially oxidized during manufacture. The oxidation products usually are aldehyde, ketone or carboxyl functional groups.

There are two main techniques, which may be employed for the development of paper chromatograms- ascending or descending techniques. In both cases the solvent is placed in the base of a sealed tank or glass jar to allow the chamber to become saturated with the solvent vapour. Ascending has two advantages. Firstly, the setup required for it is very simple. Secondly, the resolution of sample by ascending technique is somewhat better as compared to the descending technique.

The gas chromatograph consists of a gas supply and regulators (pressure and flow control), injection port, column and column oven, detector, electronics, and a data recording and processing system. The analyst must be knowledgeable about each of these GC components: carrier and detector gases; injection port temperatures and operation in split, splitless, temperature-programmed, or on-column modes; column choices and optimization (gas flows and temperature profile during separation); and detectors (TCD, FID, NPD, ECD, FPD, PFPD, and PID). The characteristics of these GC components and an understanding of basic chromatographic theory are essential to balancing the properties of resolution, capacity, speed, and sensitivity.

HPLC is a chromatographic technique of great versatility and analytical power. A basic HPLC system consists of a pump, injector, column, detector, and data system. The pump delivers mobile phase through the system. An injector allows sample to be placed into the flowing mobile phase for introduction onto the column. The HPLC column consists of stainless steel or polymer hardware filled with a separation packing material. Various auxiliary columns, particularly guard columns, may be used prior to the analytical column.

Detectors used in HPLC include UV-Vis absorption, fluorescence, RI, electrochemical, and light scattering, as well as coupled analytical systems, such as a mass spectrometer. Detection sensitivity or specificity sometimes can be enhanced by chemical derivatization of the analyte. Computer-controlled data station systems offer data collection and processing capabilities, and can run the instrument when an automated system is needed. A broad variety of column packing materials have contributed greatly to the widespread use of HPLC.

14.6 Self Assessment Questions

1. What are the applications of gas chromatography?
2. Explain in detail about the high performance liquid chromatography?
3. What is paper chromatography? How paper chromatography is used in analysis of various foods?

14.7 Reference Books

1. S. Suzanne Nielsen, Food analysis, 4th edition, 2010
2. Upadhyay and Upadhyay, Biophysical chemistry, 2006
3. Clifton.E.Meloan, food analysis 3rd edition (theory and practice)

Dr .P. KIRANMAYI

Lesson - 15

APPLICATIONS OF SPECTROSCOPY

15.0 Objective

After going through this lesson you will learn:

- The concept of Spectroscopy
- Applications of Various Spectroscopic Methods
- Principles of Colorimetry

Structure

15.1 Introduction

Spectroscopy deals with the interaction of electromagnetic radiation with matter. Spectrochemical analysis, a branch of spectroscopy, encompasses a wide range of techniques used in analytical laboratories for the qualitative and quantitative analysis of the chemical composition of foods. Common spectrochemical analysis methods include UV, Vis, and IR absorption spectroscopy; molecular fluorescence spectroscopy; and NMR spectroscopy.

In each of these methods, the analyst attempts to measure the amount of radiation either absorbed or emitted by the analyte. All of these methods make use of the facts that the energy content of matter is quantized and that photons of radiation may be absorbed or emitted by matter if the energy associated with the photon equals the energy difference for allowed transitions of that

given species. The above methods differ from each other with respect to the radiation wavelengths used in the analysis or the molecular vs. atomic nature of the analyte.

Wavelength Region	Wavelength Limits	Type of spectroscopy	Usual wavelength range	Types of trasitions in chemical systems with similar energies
Gamma rays	0.01 – 1 Å	Emission	<0.1 Å	Nuclear proton/neutron arrangements
X-rays	0.1 – 10 nm	Absorption, emission, Fluorescence and diffraction	0.1 – 100 Å	Inner-shell electrons
Ultraviolet	10 – 380 nm	Absorption, emission and fluorescence	180 – 380 nm	Outer-shell electrons in atoms, bonding electrons in molecules
Visible	380 – 750 nm	Absorption, emission and fluorescence	380 – 750 nm	Outer-shell electrons in atoms, bonding electrons in molecules
Infrared	0.075 – 1000 ìm	Absorption	0.78 to 300 ìm	Vibrational position of atoms in molecular bonds
Microwave	0.1 – 100 cm	Absorption	0.75-3.75 mm	Rotational position in molecules
Radiowave	1 – 100 m	Nuclear magnetic resonance	0.6 – 10 m	Orientation of nulei in an magnetic applied field

Wave length regions, spectroscopic method, and associated transitions

15.2 Applications

15.2.1Infrared Spectroscopy

- Identification of compounds
- Assaying the rate reactions

- Studying the conformation of molecules
- Understanding interactions between molecules

15.2.2 Fluorometry

- Quantitative analysis – assay of riboflavin, thiamine hormones such as cortisol, oestroen, serotonin and dopamine, organophosphorus pesticides, tobacco smoke carcinogens, drugs such as lysergic acid and barbiturates, porphyrins, cholesterol and even some metal ions.
- Intracellular free calcium concentration assay
- Fluorescent probes and studies on membrane structure
- Assay of membrane potential
- Fluorescent microscopy

15.2.3 Atomic Absorption Spectrophotometry

- Detect the presence of much less quantities of elements with the exception of alkali and alkali earth metals
- Assay of elements in biological samples such as blood, plasma, other body fluids such as urine, saliva, cerebrospinal fluid and milk, tissues, cells, organelles, soils, plants and even in the macromolecules.

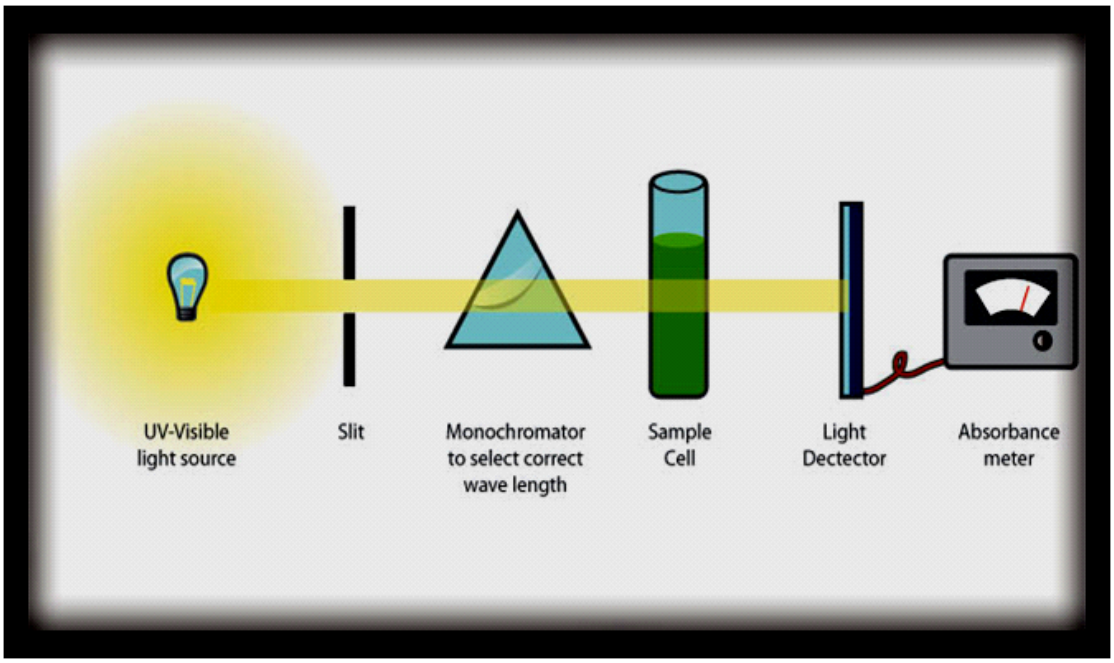
15.2.4 Nuclear Magnetic Resonance Spectroscopy

- Structural diagnosis of small organic molecules and small globular proteins
- Study of dynamic characteristics of protein structure include histones, cytochrome b_5, prothrombin, plasminogen and chromogranin A
- Studies on complex formation such as the binding of a ligand to an enzyme, a drug to DNA, an agonist to the receptor, or an antigen to an antibody
- Biological structures and compartments to study the conformation of the lipid head groups of the biological membranes and their interaction with integral proteins of the membrane and to measure intracellular pH, membrane transport phenomena, and metabolite concentrations, interconversions and fluxes
- Thermodynamic studies made on folding and unfolding of proteins and t RNAs, and partition coefficients for the distribution of a molecule between different compartments.

15.3 Colorimetry

Chromogens are chemicals that, upon reaction with the compound of interest, form a colored product. Chromogens are available that selectively react with a wide variety of minerals. Each chromogen reacts with its corresponding mineral to produce a soluble coloured product that can be quantified by absorption of light at a specified wavelength. The relationship between concentration and absorbance is given by Beer's law. Generally, concentration of mineral in a sample is determined from a standard curve developed during the analysis, although in some cases it is possible to directly calculate concentration based on molar absorptivity of the chromogen–mineral complex.

Samples generally must be ashed or treated in some other manner to isolate and/or release the minerals from organic complexes that would otherwise inhibit their reactivity with the chromogen. The mineral of interest must be solubilized from a dry ash and subsequently handled in a manner that prevents its precipitation. The soluble mineral may need to be treated (e.g., reduced or oxidized) to ensure that all mineral is in a form that reacts with the chromogen. Ideally, the chromogen reacts rapidly to produce a stable product. This is not always the case in practice, and time constraints may be established for color development and reading of absorbance. As with all mineral analysis of food, special efforts must be put in place to avoid contamination during sampling and analysis.



colorimeter

15.3.1 Applications

- Colorimetry is used for the detection and quantification of a wide variety of minerals in food, and it is often a viable alternative to atomic absorption spectroscopy and other mineral detection methods.
- Colorimetric methods generally are very specific and usually can be performed in the presence of other minerals, thereby avoiding extensive separation to isolate the mineral of interest. They are particularly robust and often immune to matrix effects that can limit the usefulness of other methods for mineral analysis.
- With minimal effort and expense, many colorimetric methods will perform with precision and accuracy similar to that obtained by experienced personnel using atomic absorption spectroscopy.

15.4 Flame Spectrophotometry

The flame photometric analysis method is more or less similar to that of spectrophotometry with the exception that the place of the sample cell is taken by a flame. Consequently, it is the

absorption or emission of specific wavelengths by excited atoms that is studied by this technique. The optical system and even the photo-detectors used in spectrophotometry are identical.

The general method of flame photometry can be applied in two complementary ways: emission flame photometry and atomic absorption spectrophotometry.

Volatilization of molecules in a flame produces free atoms and then excites them to higher energy levels. The characteristic emission spectrum of the element is produced when the excited atoms return to their ground state. This is the principle of emission flame photometry. Atomic absorption spectrophotometry, on the other hand, measures the absorption of a beam of monochromatic light by atoms in a flame. Since the transitions available to the electrons in any given atom are specified by the available energy levels, atomic spectra are absolutely specific for the element involved. Moreover, the energy absorbed are emitted is proportional to the number of atoms present in the optical path. Thus, apart from giving the identity of the element present in a sample, flame photometry also provides information about the quantity of the elements present.

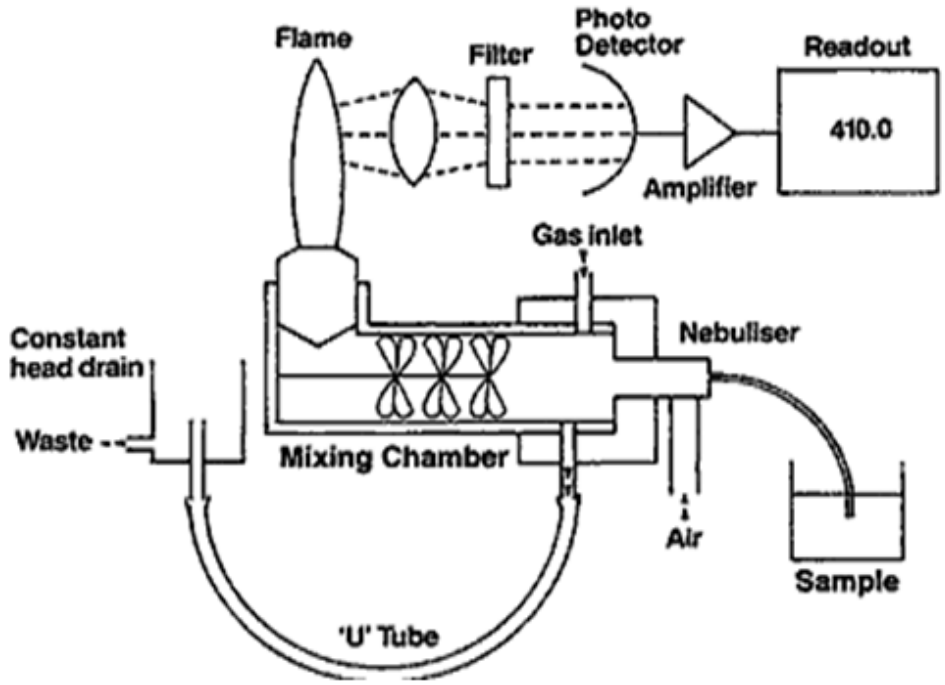
The amount of energy emitted also depends upon the temperature and composition of the flame. It is therefore very necessary that the two flame variables must be maintained constant and that standard solutions be used to calibrate the system. The need to maintain flame composition constant also dictates which element should be determined first. Thus, sodium which gives a very high background emission is measured first and the quantity of the sodium determined is added to all the standards. Certain elements, such as the alkali metals, enhance the emission of the other elements. On the other hand, some substances like aluminate and silicate cause a decrease in emission of other elements. To relieve this deleterious effect certain other elements known as the releasing agents (strontium or lanthanum) must be added.

Another way to deal with interferences is to measure all interfering components in a given sample. After this, standards for each of the components are prepared which contain the previously determined concentration of interfering components. This is known as cyclical analysis. About two to three such cycles are necessary before an accurate idea of the quantities of all sample components can be determined with a fair degree of accuracy.

Organic material in biological samples (which might cause interference) is usually removed by ashing. To prevent more volatile elements from getting sublimated, the ashing is usually carried under low temperatures in the presence of oxygen. Alternatively, liquid ashing, i.e., oxidating digestion of sample in hydrogen peroxide/concentrated sulfuric acid solution may be carried out. A small amount of selenium sulphate, if added, acts as a catalyst, while lithium sulphate is sometimes added to raise the boiling point.

It is advisable to store samples and standards in polythene bottles. This is so since even the good quality glass containers release metal ions. Flame instability can cause large errors in flame photometric analysis. It is, therefore, essential that all assays be carried out in triplicate. Calibration curves should be checked or reconstructed when the assays are carried out. If high accuracy is desired, a standard solution containing more or less the same concentration of the element as the sample solution is assayed immediately before and after the sample solution. This method is knowing as bracketing. Very frequently, internal standards are used and the choice element for the purpose is lithium.

15.4.1 Instrumentation



Basic components of an emission flame photometer

15.4.1.a. Nebulizers:

Samples, before they get in to the flame, must be converted into a fine spray i.e., nebulized. This is necessary because large drops do not remain in the hottest part of the flame for a long time and therefore may not become volatilized and excited. Because of this reason, the nebulizer becomes the most critical part of a flame photometer so much so that the efficiency of an analysis depends on the efficiency of the nebulizer.

Simple nebulizers are essentially of the 'scent spray' type where by a forced stream of air (travelling at nearly the speed of sound) passes over a capillary tube dipping in to the test solution. This leads to a considerable drop in pressure leading to a suction of the sample through the capillary. As sample emerges from the tip of the capillary it is broken in to a mist of fine droplets by the flow of air. To eliminate large drops, a cloud chamber is placed between the flame and the nebulizer where the large drops condense and drain away.

Since large droplets condense the drain away, there is a large loss of sample (90% is lost, only 10% reaches the flame) and if one has only a small amount of sample to start with, this poses a big problem. To alleviate this problem, an improvement on the basic design of the nebulizer has been achieved by placing an impact bead a few millimeters away from the nebulizer tip. The large droplet, when they emerge from the tip, collide with the bead and broken in to smaller droplets.

This design lowers the average droplet size and thus improves the efficiency of the analysis besides reducing the loss of sample.

15.4.1.b The Flame: Various gas mixtures producing different flames differing in their temperatures are used in flame photometry.

1.5.4.1.c Monochromators: In sophisticated instruments prisms are sometimes even diffraction gratings are used. However, for routine analysis of such elements as calcium, sodium and potassium a simple filter might suffice.

15.4.1.d Photocells: These are the usual detectors in a flame photometer. Unfortunately the flame instability reduces their accuracy. Therefore a multi channel polychromator is used in some routine procedures to allow measurement of up to six elements simultaneously.

15.4.2 Applications

- The primary use of flame photometry is in the assay of elements in biological samples such as blood, plasma, other body fluids such as urine, saliva, cerebrospinal fluid, milk, tissues, cells, organelles, soils, plants and even in the micro molecules.
- Flame photometry is used in routine estimation of sodium, potassium, calcium, magnesium, manganese, indium and thallium in a host of biological samples. It is a very sensitive to the estimation of alkali, alkaline- earth and rare earth elements.
- It also permits the estimation of silver, aluminium, gold, bismuth, cadmium, copper, mercury, lead, selenium, iron and zinc. Although most elemental analysis is possible directly copper, iron, lead and mercury need to be extracted from the biological fluids before they can be assayed.

15.5 Summary

Spectroscopy deals with the interaction of electromagnetic radiation with matter. Common spectrochemical analysis methods include UV, Vis, and IR absorption spectroscopy; molecular fluorescence spectroscopy; and NMR spectroscopy. Chromogens are chemicals that, upon reaction with the compound of interest, form a colored product. Chromogens are available that selectively react with a wide variety of minerals. Each chromogen reacts with its corresponding mineral to produce a soluble coloured product that can be quantified by absorption of light at a specified wavelength.

Colorimetry is used for the detection and quantification of a wide variety of minerals in food, and it is often a viable alternative to atomic absorption spectroscopy and other mineral detection methods. The flame photometric analysis method is more or less similar to that of spectrophotometry with the exception that the place of the sample cell is taken by a flame.

15.6 Self Assessment Questions

1. What are the applications of various spectroscopic methods?
2. What are the principles of colorimetry?
3. Explain the instrumentation of flame photometer?

15.7 Reference Books:

1. S. Suzanne Nielsen, Food analysis, 4th edition, 2010
2. Upadhyay and Upadhyay, Biophysical chemistry, 2006
3. Clifton.E.Meloan, food analysis 3rd edition (theory and practice)

Dr.P. KIRANMAYI

Lesson - 16

CARBOHYDRATE ANALYSIS

16.0 Objective

After going through this lesson you will learn:

- Analysis of total carbohydrates
- Specific analysis of monosaccharides
- Analysis of total starch

Structure

16.1 Introduction

Carbohydrates are important in foods as a major source of energy, to impart crucial textural properties, and as dietary fiber which influences physiological processes. Digestible carbohydrates, which are converted into monosaccharides, which are absorbed, provide metabolic energy. Worldwide, carbohydrates account for more than 70% of the caloric value of the human diet. Most of the carbohydrate calories should come from starch. Nondigestible polysaccharides (all those other than starch) comprise the major portion of dietary fiber.

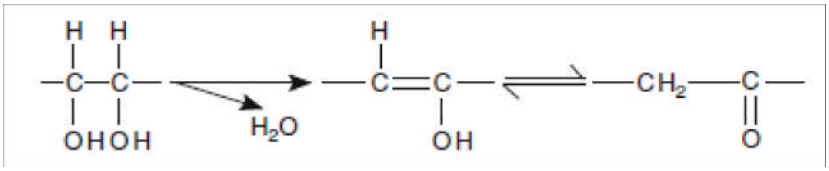
Ingested carbohydrates are almost exclusively of plant origin, with milk lactose being the major exception. Of the monosaccharides (sometimes called simple sugars), only D-glucose and D-fructose are found in other than minor amounts. Monosaccharides are the only carbohydrates that can be absorbed from the small intestine. Higher saccharides (oligo- and polysaccharides) must first be digested (i.e., hydrolyzed to monosaccharides) before absorption and utilization can occur.

In general, evolution of analytical methods for carbohydrates has followed the succession: qualitative colour tests, adaptation of the colour test for reducing sugars based on reduction of Cu(II) to Cu(I) (Fehling test) to quantitation of reducing sugars, qualitative paper chromatography, quantitative paper chromatography, gas chromatography (GC) of derivatized sugars, qualitative and quantitative thin layer chromatography, enzymic methods, and high performance liquid chromatography (HPLC).

16.2 Total Carbohydrate

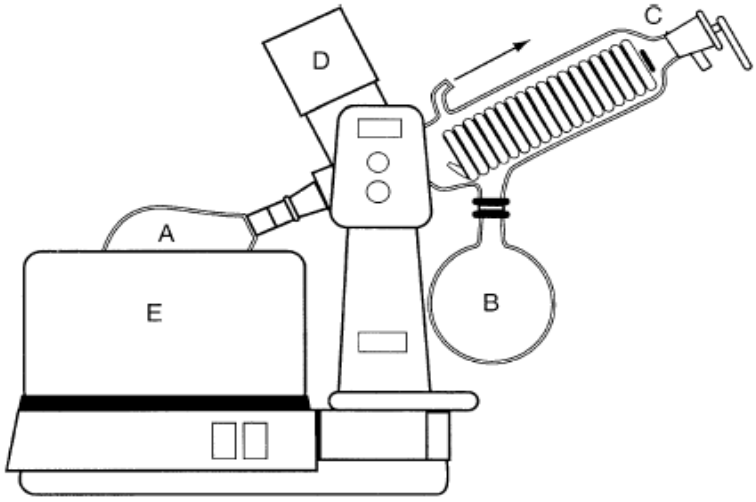
16.2.1 Phenol-Sulfuric Acid Method

Carbohydrates are destroyed by strong acids and/or high temperatures. Under these conditions, a series of complex reactions takes place, beginning with a simple dehydration reaction as shown in Equation.



Continued heating in the presence of acid produces various furan derivatives. These products then condense with themselves and other products to produce brown and black substances. They will also condense with various phenolic compounds, such as phenol, resorcinol, orcinol, α-naphthol, and napthoresorcinol, and with various aromatic amines, such as aniline and *o*-toluidine, to produce colored compounds that are useful for carbohydrate analysis.

The most often used condensation is with phenol itself. Virtually all classes of sugars, including sugar derivatives and oligo- and polysaccharides, can be determined with the phenol-sulfuric acid method.



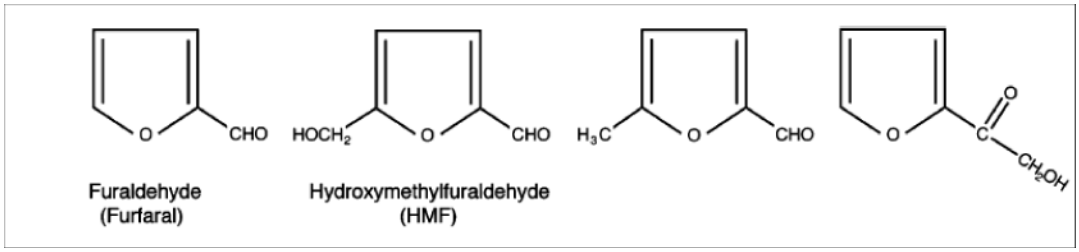
Rotary evaporator

Round bottom flask A; condensate collects in flask B; condenser C; motor D; water bath E

16.3 Total Reducing Sugar

16.3.1 Somogyi–Nelson Method

Oxidation is a loss of electrons; reduction is a gain of electrons. Reducing sugars are those sugars that have an aldehyde group (aldoses) that can give up electrons (i.e., act as a reducing agent) to an oxidizing agent, which is reduced by receiving the electrons. Oxidation of the aldehydo group produces a carboxylic acid group. Under alkaline conditions, ketoses behave as weak reducing sugars because they will partially isomerize to aldoses. The most often used method to determine amounts of reducing sugars is the Somogyi–Nelson method, also at times referred to as the Nelson–Somogyi method. The Somogyi–Nelson method is based on the reduction of Cu(II) ions to Cu(I) ions by reducing sugars. The Cu(I) ions then reduce an arsenomolybdate complex, prepared by reacting ammonium molybdate [$(NH_4)_6Mo_7O_{24}$] and sodium arsenate (Na_2HAsO_7) in sulfuric acid. Reduction of the arsenomolybdate complex produces an intense, stable blue color that is measured spectrophotometrically.



Furan products that could arise from, in order, pentoses and hexuronic acids, hexoses, 6-deoxyhexoses and ketohexoses

16.4 Specific Analysis of Mono and Oligosaccharides

16.4.1 High-performance Liquid Chromatography

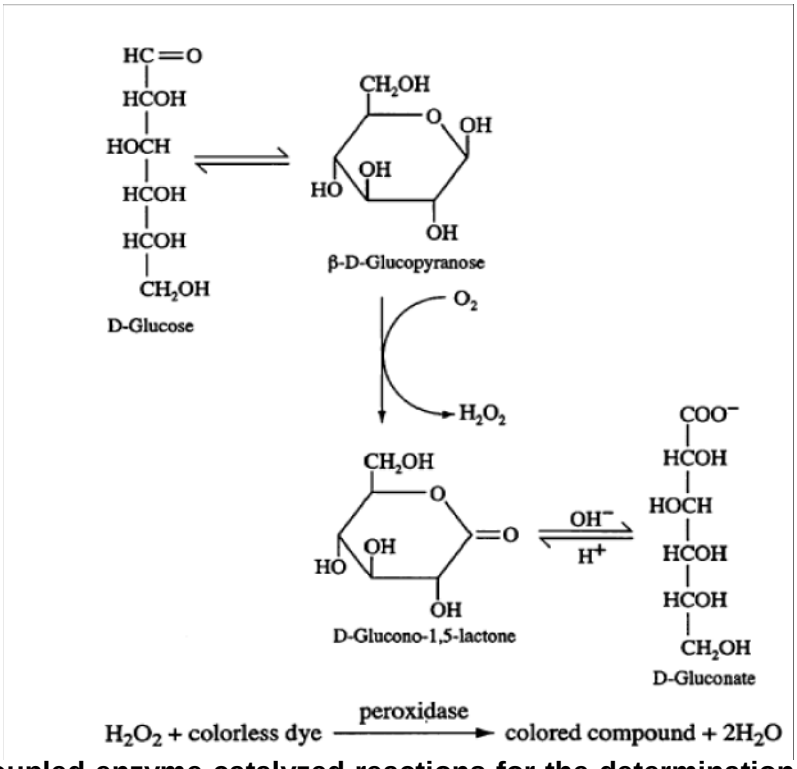
HPLC is the method of choice for analysis of mono- and oligosaccharides and can be used for analysis of polysaccharides after hydrolysis. HPLC gives both qualitative analysis (identification of the carbohydrate) and, with peak integration, quantitative analysis. HPLC analysis is rapid, can tolerate a wide range of sample concentrations, and provides a high degree of precision and accuracy. HPLC requires no prior derivatization of carbohydrates, unlike GC of sugars, but does require micron filter filtration prior to injection. Complex mixtures of mono- and oligosaccharides can be analyzed.

16.4.2 Gas Chromatography

GC (gas-liquid chromatography, GLC), provides both qualitative and quantitative analysis of carbohydrates. For GC, sugars must be converted into volatile derivatives. The most commonly used derivatives are the alditol peracetates (and aldonic acid pertrimethylsilyl ethers from uronic acids). A flame ionization detector is the detector of choice for peracetylated carbohydrate derivatives. The most serious problem with GC for carbohydrate analysis is that two preparation steps are involved: reduction of aldehyde groups to primary alcohol groups and conversion of the reduced sugar into a volatile peracetate ester or pertrimethylsilyl ether derivative.

16.5 Enzymic Determination of D-Glucose

The enzyme glucose oxidase oxidizes D-glucose quantitatively to D-glucono-1, 5-lactone (glucono-deltalactone), the other product being hydrogen peroxide.



Coupled enzyme-catalyzed reactions for the determination of D-glucose

To measure the amount of D-glucose present, peroxidase is added along with a colorless compound that can be oxidized to a colored compound. In a second enzyme-catalyzed reaction, the leuco dye is oxidized to a colored compound which is measured spectrophotometrically. Various dyes are used in commercial kits. The method using this combination of two enzymes and an oxidizable colorless compound is known as the GOPOD (glucose oxidaseperoxidase) method.

16.6 Polysaccharides

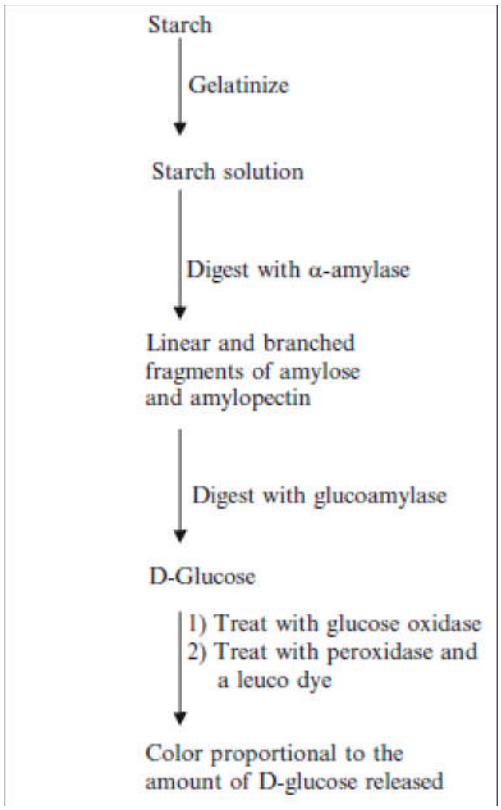
16.6.1 Starch

Starch is second only to water as the most abundant component of food. Starch is found in all parts of plants (leaves, stems, roots, tubers, seeds). A variety of commercial starches are available worldwide as food additives. These include corn (maize), waxy maize, high-amylose corn (amylomaize), potato, wheat, rice, tapioca (cassava), arrowroot, and sago starches. In

addition, starch is the main component of wheat, rye, barley, oat, rice, corn, mung bean and pea flours and certain roots and tubers such as potatoes, sweet potatoes and yams.

16.6.2 Total Starch

The only reliable method for determination of total starch is based on complete conversion of the starch into D-glucose by purified enzymes specific for starch and determination of the D-glucose released by an enzyme specific for it.

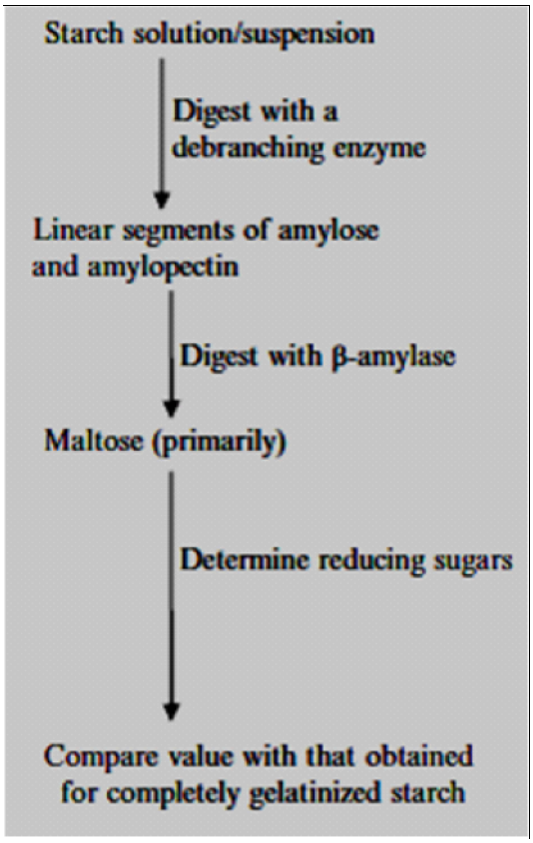


Flow diagram of determination total starch

16.6.3 Degree of Gelatinization of Starch

When starch granules are heated in water to a temperature specific for the starch being cooked, they swell, lose their crystallinity and birefringence, and become much more susceptible to enzyme-catalyzed hydrolysis. Heating starch in water produces phenomena that result from two processes: gelatinization and pasting, often together referred to simply as gelatinization, which are very important in determining the texture and digestibility of foods containing starch.

Several methods have been developed that make use of the fact that certain enzymes act much more rapidly on cooked starch than they do on native starch. A particularly sensitive method employs a combination of pullulanase and β-amylase, neither of which is able to act on uncooked starch granules. With gelatinized or pasted starch, the enzyme pullulanase debranches amylopectin and any branched amylase molecules, giving a mixture of linear segments of various sizes. (Another debranching enzyme, isoamylase, may also be used.) β-Amylase then acts on the linear chains, releasing the disaccharide maltose, starting at the nonreducing ends and a small amount of maltotriose (from chains containing an odd number of glucosyl units). The degree of gelatinization is determined by measuring the amount of reducing sugar formed.



Flow diagram of determination of degree of gelatinization of starch

16.6.4 Degree of Retrogradation of Starch

Upon storage of a product containing cooked starch, the two starch polymers, amylose and amylopectin, associate with themselves and with each other, forming polycrystalline arrays. This process of reordering is called retrogradation. (Retrogradation is a contributing factor to the staling of bread and other bakery products, for example.) Retrograded starch, like native starch, is acted on very slowly by the combination of pullulanase plus β-amylase. The decrease in reducing power (from maltose released by action of the enzyme combination) after storage is a measure of the amount of retrograded starch at the time of analysis and/or the degree of retrogradation.

16.7 Pectin Content Determination

The constant in pectins is D-galacturonic acid as the principal component (often at least 80%). However, glycosidic linkages of uronic acids are difficult to hydrolyze without decomposition, so methods involving acid catalyzed hydrolysis to release D-galacturonic acid and chromatography are generally not applicable. One method employed for pectin uses saponification in sodium hydroxide solution, followed by acidification, and addition of Ca^{2+} to precipitate the pectin. Calcium pectate is collected, washed, dried, and measured gravimetrically. Precipitation with the quaternary ammonium salt cetylpyridinium bromide has been used successfully because there is a much lower critical electrolyte concentration for its salt formation with pectin than with other acidic polysaccharides, and because pectin and other acidic polysaccharides are not likely to be found together. Because of the dominance of D-galacturonic acid in its structure, pectins are often determined using the carbazole or *m*-hydroxydiphenyl methods. Isolation of crude pectin usually precedes analysis.

16.8 Degree of Esterification

The degree of esterification (DE) is a most important parameter in both natural products and added pectin. DE may be measured directly by titration before and after saponification. First, the isolated pectin is washed with acidified alcohol to convert carboxylate groups into free carboxylic acid groups and then washed free of excess acid. Then, a dispersion of the pectinic acid in water is titrated with dilute base, such as standardized sodium hydroxide solution, to determine the percentage of nonesterified carboxyl ester groups. Excess base is added to saponify the methyl ester groups. Back-titration with standardized acid to determine excess base following saponification gives the DE.

16.9 Summary

For determination of low-molecular-weight carbohydrates, older colorimetric methods for total carbohydrate, various reducing sugar methods, and physical measurements have largely been replaced by chromatographic methods. The older chemical methods suffer from the fact that they are not stoichiometric and, therefore, require standard curves. This makes them particularly problematic when a mixture of sugars is being determined. Physical measurements are not specific for carbohydrates. Chromatographic methods (HPLC and GC) separate mixtures into the component sugars, identify each component by retention time, and provide a measurement of the mass of each component.

Enzymatic methods are specific and sensitive, but seldom, except in the case of starch, is determination of only a single component desired. HPLC is widely used for identification and measurement of mono- and oligosaccharides. Polysaccharides are important components of

many food products. Yet there is no universal procedure for their analysis. Generally, isolation must precede measurement. Isolation introduces errors because no extraction or separation technique is stochiometric. Identification and measurement are done by hydrolysis to constituent monosaccharides and their determination. An exception is starch, which can be digested to glucose using specific enzymes (amylases), followed by measurement of the glucose released.

16.10 Self Assessment Questions

1. How to estimate total reducing sugars?
2. Explain the specific analysis of mono and oligosaccharides?
3. What is degree of gelatinization?
4. Explain the degree of esterification.

16.11 Reference Books:

1. S. Suzanne Nielsen, Food analysis, 4th edition, 2010
2. Upadhyay and Upadhyay, Biophysical chemistry, 2006
3. Clifton.E.Meloan, food analysis 3rd edition (theory and practice)

Dr. P. KIRANMAYI

Lesson - 17

DIETARY FIBER

17.0 Objective

After going through this lesson you will learn:

- The importance of dietary fiber
- Major components of dietary fiber
- Various methods used for estimation of dietary fiber

Structure

17.1 Introduction

Dietary fiber is essentially the sum of the nondigestible components of a foodstuff or food product. Most, but not all, dietary fiber is plant cell-wall material (cellulose, hemicelluloses, lignin) and thus is composed primarily of polysaccharide molecules. Because only the amylase and amylopectin molecules in cooked starch are digestible, all other polysaccharides are also components of dietary fiber. Some are components of insoluble fiber; some make up soluble fiber. Insoluble dietary fiber components are cellulose, microcrystalline cellulose added as a food ingredient, lignin, hemicelluloses entrapped in a lignocellulosic matrix, and resistant starch. Other polysaccharides, including many, but not all, hemicelluloses not entrapped in a lignocellulosic matrix, much of the native pectin, and the majority of hydrocolloids/ food gums, are classified as soluble dietary fiber.

Measurement of insoluble fiber is important not only in its own right, but also for calculating the caloric content of a food. According to nutrition labeling regulations, one method allowed to calculate calories involves subtracting the amount of insoluble dietary fiber from the value for total carbohydrate, before calculating the calories based on protein, fat, and carbohydrate content (approximately 4, 9, and 4 Calories per gram, respectively). This method ignores the fact that soluble fiber, like insoluble fiber, is also essentially noncaloric. [Fiber components can contribute calories via absorption of products of fermentation (mostly short-chain fatty acids).

17.2 Definition

Dietary fiber is the part of whole grains, fruits, vegetables, beans, nuts, and seeds that we cannot digest. Dietary fiber is not a single substance, but a group of substances with similar properties. Some of the components of fiber are cellulose, hemicellulose, lignin, gum, and pectin. Dietary fiber can be separated into two basic types based on its properties and effects on the body. These two types are insoluble and soluble fiber. Insoluble fibers, such as cellulose, hemicellulose, and lignin, do not dissolve in water. Insoluble fibers are found in foods such as wheat bran, whole grains, and vegetables. Insoluble fibers absorb water and increase the intestinal bulk, which helps the intestine function properly. Soluble fibers, such as gum and pectin, dissolve in water and are found in beans, oats, barley, and some fruits and vegetables. Soluble fibers may play a role in lowering blood cholesterol and in regulating the body's use of sugar.

Gum	**pectin**
Oatmeal	Squash
Rolled oat	Apples
products	Citrus fruits
Dried beans	
Cauliflower	
Green beans	
Cabbage	
Dried peas	
Carrots,potatoes	
strawberries	

Food sources of soluble fiber

Food sources of insoluble fiber

Cellulose	Hemicelluloses	Lignin
Whole wheat flour	Bran cereals	Bran cereals
Unprocessed bran	Whole grains	Unprocessed bran
Cabbage	Brussels sprouts	Strawberries
Peas	Mustard greens	Eggplant
Green beans	Beet root	Pears
Wax beans		Green beans
Broccoli		radishes
Brussel sprouts		
Cucumber with skin		
Green peppers		
Apples		
carrots		

17.3 Importance of Dietary Fiber

Adequate consumption of dietary fiber from a variety of foods will help protect against colon cancer and also help to keep blood lipids within the normal range, thereby reducing the risk of obesity, hypertension, and cardiovascular disease in general. Certain types of fiber can slow D-glucose absorption and reduce insulin secretion, which is of great importance for diabetics and probably contributes to the well-being of nondiabetics as well. Dietary fiber includes a variety of materials that in turn produce a variety of physiological actions. For example, the pentosan fraction of dietary fiber seems to be most beneficial in preventing colon cancer and reducing cardiovascular disease. Pectin and the hydrocolloids are most beneficial in slowing glucose absorption and in lowering insulin secretion. A mixture of hemicelluloses and cellulose will help prevent diverticulosis and constipation.However, dietary fiber is not a magic potion that will correct or prevent all diseases. Rather, dietary fiber is an essential component of a well-balanced diet that will help minimize some common health problems.

17.4 Major Components of Dietary Fiber

The major components of natural dietary fiber are cellulose, hemicelluloses, lignin, and other nonstarch plant polysaccharides such as pectin. In a food product, added hydrocolloids/food gums, resistant starch, and certain oligosaccharides such as those derived from inulin are included because they are also nondigestible and provide certain of the physiological benefits of dietary

fiber. An example is polydextrose, which is often, but not always, used in product formulations specifically because it is considered to be soluble dietary fiber.

17.5 Methods

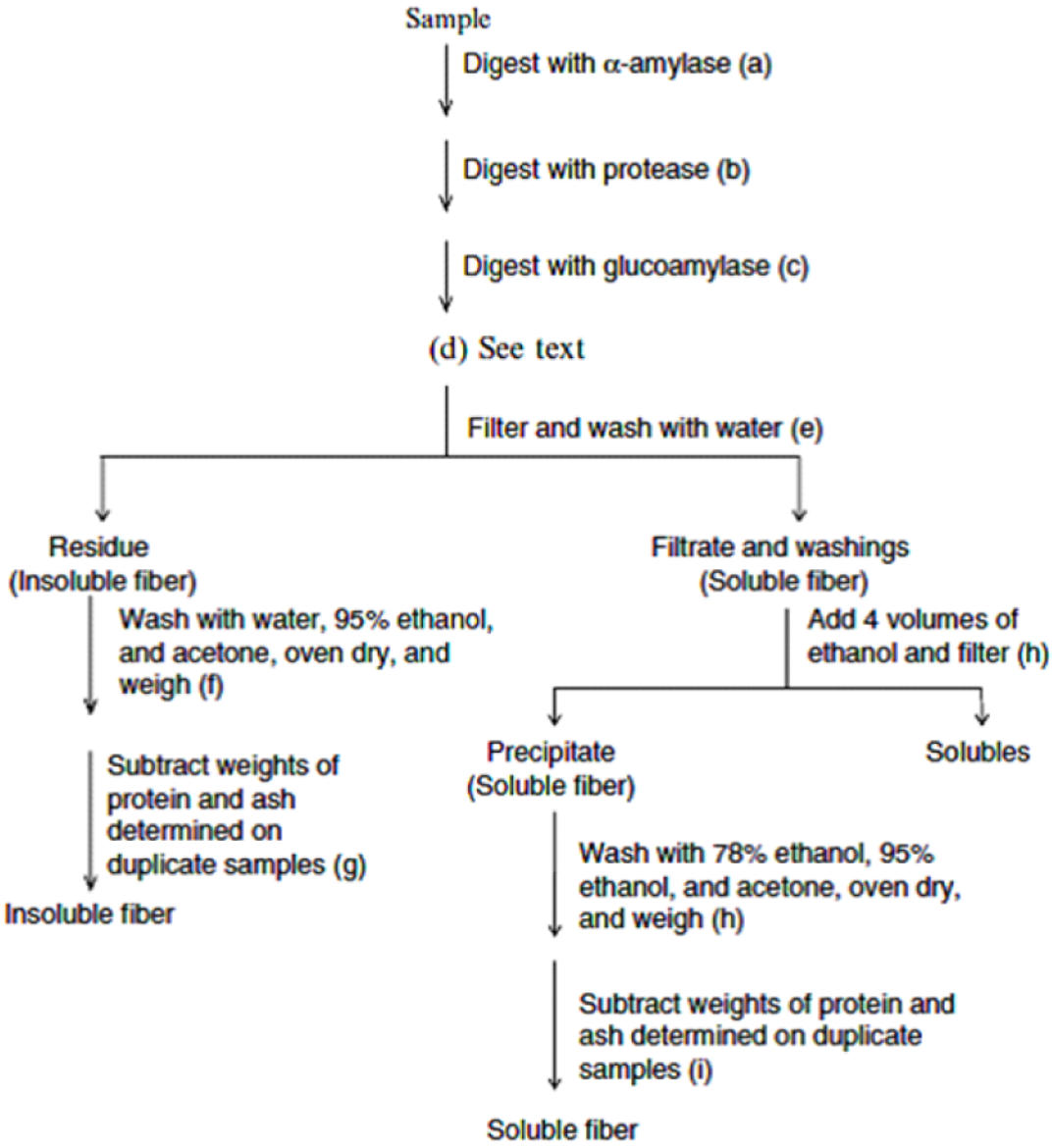
Dietary fiber is often determined gravimetrically. In such a procedure, digestible carbohydrates, lipids, and proteins are selectively solubilized by chemicals or removed by enzyme-catalyzed hydrolysis. Then, non solubilized and/or non digested materials are collected by filtration, and the fiber residue is recovered, dried, and weighed. The food component that may be most problematic in fiber analysis is starch. In any method for determination of dietary fiber, it is essential that all digestible starch be removed, for incomplete removal of digestible starch increases the residue weight and inflates the estimate of fiber. Alpha-amylase, debranching enzymes, and glucoamylase (amyloglucosidase) are enzymes used in starch analysis α-Amylase catalyzes hydrolysis of unbranched segments of 1,4-linked α -D-glucopyranosyl units forming primarily alto oligosaccharides composed of 3–6 units. Debranching enzymes (both pullulanase and isoamylase are used) catalyze hydrolysis of the 1, 6 linkages that constitute the branch points and thereby produce short linear molecules. Glucoamylase (amyloglucosidase) starts at the nonreducing ends of starch chains and releases D-glucose, one unit at a time; it will catalyze hydrolysis of both 1, 4 and 1, 6 α -D-glucosyl linkages.

All fiber methods include a heating step (95–100 ^{0}C for 35min) to gelatinize starch granules and make them susceptible to hydrolysis. Resistant starch molecules remain unhydrolyzed and, therefore, are usually measured as dietary fiber, but not all nondigestible products made from starch may be determined as dietary fiber by the approved methods. Nondigestible oligosaccharides such as those derived from inulin and certain specially prepared maltodextrins also are problematic in an analytical sense since they are in the soluble portion that is not precipitated with ethanol. It is essential either that all digestible materials be removed from the sample so that only nondigestible polysaccharides remain or that the nondigestible residue be corrected for remaining digestible contaminants.

Lipids are removed easily from the sample with organic solvents and generally do not pose analytical problems for the fiber analyst. Protein and minerals that are not removed from the sample during the solubilization steps should be corrected for by Kjeldahl nitrogen analysis and by ashing portions of the fiber residue. Because labeling of dietary fiber content is required, because dietary fiber is a complex heterogeneous material containing several substances with different solubilities and other properties, and because of its physiological importance, methods for fiber determination continue to be researched and refined. The most widely used general method for total, soluble, and insoluble dietary fiber AOACMethod 991.43 is outlined below.

17.5.1 AOAC Method 991.43

This method determines soluble, insoluble, and total dietary fiber in cereal products, fruits and vegetables, processed foods, and processed food ingredients. Starch and protein are removed from a sample by treating the sample sequentially with a thermostable α-amylase, a protease, and glucoamylase (amyloglucosidase).



Flow diagram of AOAC method for 991.43 for determining soluble, insoluble, and total dietary fiber

The insoluble residue is recovered and washed (insoluble dietary fiber). Ethanol is added to the soluble portion to precipitate soluble polysaccharides (soluble dietary fiber). To obtain total dietary fiber (TDF), the alcohol is added after digestion with the glucoamylase, and the soluble and insoluble dietary fiber fractions are collected together, dried, weighed, and ashed.

17.5.2 Physical Methods

17.5.2.a Microscopy

Microscopy can be a valuable tool in food analysis. Various kinds of microscopy [light, fluorescence, confocal scanning laser (CSLM), Fourier transforminfrared (FTIR), scanning electron (SEM), and transmission electron (TEM) microscopies] have been used to study the organization of food products and the stability of emulsions and foams and to identify extraneous matter and its amount. Microscopy is particularly useful in examinations of starchy foods. Granule size, shape, and form, the birefringence endpoint temperature determined using a polarizing microscope with a hot stage, and, in some cases, iodine-staining characteristics can be used to identify the starch source.

In cooked starch products, the extent of retrogradation and the effects of storage on microstructure have been evaluated by iodine staining and light microscopy. The degree that starch has been damaged mechanically during dry milling, the extent of digestion by enzymes, and whether the starch-based product has been overcooked, undercooked, or correctly cooked also can be determined microscopically. Quantitative microscopy has been employed for analysis of the nonstarch polysaccharides of cereal grains.

17.5.2.b Mass and NIR Transmittance Spectrometry

Mass and NIR transmittance spectrometry have been used to determine sugar content.

17.5.2.c Specific Gravity

Specific gravity is defined as the ratio of the density of a substance to the density of a reference substance (usually water), both at a specified temperature. The concentration of a carbohydrate solution can be determined by measuring the specific gravity of the solution, then referring to appropriate specific gravity tables. Measurement of specific gravity as a means of determining sugar concentration is accurate only for pure sucrose or other solutions of a single pure substance, but it can be, and is, used for obtaining approximate values for liquid products. Two basic means of determining specific gravity are used. By far the most common is use of a hydrometer calibrated either in Brix, which corresponds to sucrose concentrations by weight. The values obtained are converted into concentrations by use of tables constructed for the substance in the pure solution, e.g., sucrose or glucose syrups.

17.5.2.d Refractive Index

When electromagnetic radiation passes from one medium to another, it changes direction (i.e., is bent or refracted). The ratio of the sine of the angle of incidence to the sine of the angle of refraction is termed the refractive index (RI). The RI varies with the nature of the compound, the temperature, the wavelength of light, and the concentration of the compound. By holding the first three variables constant, the concentration of the compound can be determined by measuring the RI. Thus, measurement of refractive index is another way to determine total solids in solution. Like determination of specific gravity, use of RI to determine concentrations is accurate only for pure sucrose or other solutions of a single pure substance, and also like the determination of specific gravity, it is used for obtaining approximate sugar concentrations in liquid products. In this case, the solution must be clear. Refractometers that read directly in sucrose units are available.

Along with the above methods gravimetric methods for dietary fiber can be divided into detergent methods and enzymatic methods. The latter may determine insoluble fiber only or insoluble plus soluble fiber. The acid detergent fiber (ADF) method, which determines cellulose and lignin content, has been accepted by the AOAC as a method of testing feed stuffs. The neutral detergent fiber method (NDF) is an official assay for the determination of dietary fiber in cereal grains. It measures cellulose, hemicelluloses and lignin. Both detergent methods measure only detergent insoluble dietary fiber. This insoluble dietary fiber can be separated into cellulose, hemicelluloses and lignin. Determination of hemicelluloses from the difference is not precise because of the presence of various components in the detergent methods. Some of the error can be reduced by a sequential analysis of NDF and ADF. Pectins and tannins are soluble in the NDF solution and hemicelluloses and can be estimated from the weight loss of starch and protein-free NDF residue after ADF treatment.

Enzymatic methods are designed to measure dietary fiber under physiological conditions. Some enzymatic preparations may contain adventitious foreign activities and my degrade to a limited extent dietary fiber compo9nents. On the other hand, some protein may be incompletely degrade to a limited 0065tent dietary fiber components. On the other hand, some protein may be incompletely degraded and attached or bound to the separated fiber. For routine analysis in quality control, for food labeling, and for separate determination of soluble and insoluble fiber, the enzymatic-gravimetric approach is generally preferred.

17.6 Summary

Insoluble dietary fiber, soluble dietary fiber, and total dietary fiber are each composed primarily of nonstarch polysaccharides. The method for the determination of starch is based on its complete conversion to, and determination of, D-glucose. Methods for the determination of total dietary fiber and its components rely on removal of the digestible starch in the same way and often on removal of digestible protein with a protease, leaving non digestible components. Dietary fiber is often determined gravimetrically. In such a procedure, digestible carbohydrates, lipids, and proteins are selectively solubilized by chemicals or removed by enzyme-catalyzed hydrolysis The most widely used general method for total, soluble, and insoluble dietary fiber AOAC Method 991.43'.

17.7 Self Assessment Questions

1. What is dietary fiber?
2. What is the importance of dietary fiber?
3. What are the major components of dietary fiber?
4. Explain about AOAC method for estimation of dietary fiber.
5. What are the various physical methods used in analysis of dietary fiber?

17.8 Reference Books:

1. S. Suzanne Nielsen, Food analysis, 4th edition, 2010
2. Upadhyay and Upadhyay, Biophysical chemistry, 2006
3. Clifton.E.Meloan, food analysis 3rd edition (theory and practice)

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LESSON - 18

FAT ANALYSIS

18.0 Objective

After going through this lesson you will learn:

- Solvent extraction methods
- Semicontinuous solvent extraction methods
- Discontinuous solvent extraction methods
- Nonsolvent wet extraction methods

Structure

18.10 Self Assessment Questions

18.11 Reference Books

18.1 Introduction

Lipids, proteins, and carbohydrates constitute the principal structural components of foods. Lipids are a group of substances that, in general, are soluble in ether, chloroform, or other organic solvents but are sparingly soluble in water. Some lipids, such as triacylglycerols, are very hydrophobic. Other lipids, such as di- and monoacylglycerols, have both hydrophobic and hydrophilic moieties in their molecules and are soluble in relatively polar solvents. Short-chain fatty acids such as C1–C4 are completely miscible in water and insoluble in nonpolar solvents.

Triacylglycerols are fats and oils that represent the most prevalent category of the group of compounds known as lipids. The terms lipids, fats, and oils are often used interchangeably. Fats generally refer to those lipids that are solid at room temperature and oils generally refer to those lipids that are liquid at room temperature.

18.2 Solvent Extraction Methods

The total lipid content of a food is commonly determined by organic solvent extraction methods or by alkaline or acid hydrolysis followed by Mojonnier extraction. For multicomponent food products, acid hydrolysis is often the method of choice. Both acid hydrolysis and alkaline hydrolysis methods can be performed using Mojonnier extraction equipment. The use of acid hydrolysis eliminates some of the matrix effects that may be exhibited by simple solvent extraction methods.

The accuracy of direct solvent extraction methods (i.e., without prior acid or alkaline hydrolysis) greatly depends on the solubility of the lipids in the solvent used and the ability to separate the lipids from complexes with other macromolecules. The lipid content of a food determined by extraction with one solvent may be quite different from the content determined with another solvent of different polarity. In addition to solvent extraction methods, there are nonsolvent wet extraction methods and several instrumental methods that utilize the physical and chemical properties of lipids in foods for fat content determination. For nutrition labeling purposes, total fat is most commonly determined by gas chromatography (GC) analysis.

18.2.1 Acid Hydrolysis

A significant portion of the lipids in foods such as dairy, bread, flour, and animal products is bound to proteins and carbohydrates, and direct extraction with nonpolar solvents is inefficient. Such foods must be prepared for lipid extraction by acid hydrolysis. This includes a significant percentage of finished food products. Acid hydrolysis can break both covalently and ionically bound lipids into easily extractable lipid forms. The sample can be predigested by refluxing for 1 h with 3*N* hydrochloric acid. Ethanol and solid hexametaphosphate may be added to facilitate separation of lipids from other components before food lipids are extracted with solvents. For example, the acid hydrolysis of two eggs requires 10 ml of HCl and heating in a water bath at 65æ%C for 15–25 min or until the solution is clear.

18.3 Continuous Solvent Extraction Method

18.3.1 Goldfish Method

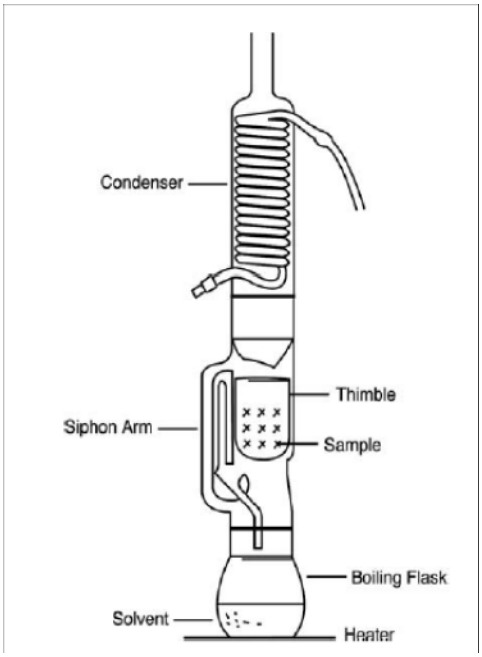
For continuous solvent extraction, solvent from a boiling flask continuously flows over the sample held in a ceramic thimble. Fat content is measured by weight loss of the sample or by weight of the fat removed. The continuous methods give faster and more efficient extraction than semicontinuous extraction methods. However, they may cause channeling which results in incomplete extraction. The Goldfish test is example of continuous lipid extraction methods.

Gold fish fat extractor

18.4 Semicontinuous Solvent Extraction

18.4.1 Soxhlet Method

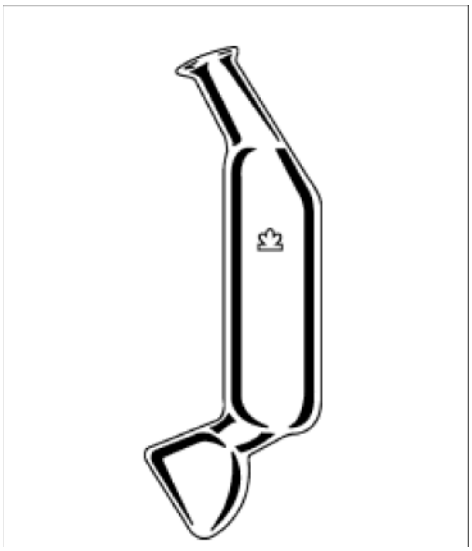
For semicontinuous solvent extraction, the solvent builds up in the extraction chamber for 5–10 min and completely surrounds the sample and then siphons back to the boiling flask. Fat content is measured by weight loss of the sample or by weight of the fat removed. This method provides a soaking effect of the sample and does not cause channeling. However, this method requires more time than the continuous method. Instrumentation for a more rapid and automated version of the Soxhlet method is used for some quality control applications.



Soxhlet extraction apparatus

18.5 Discontinuous Solvent Extraction

18.5.1 Mojonnier Method



Mojonnier fat extraction flask

Fat is extracted with a mixture of ethyl ether and petroleum ether in a Mojonnier flask, and the extracted fat is dried to a constant weight and expressed as percent fat by weight. The Mojonnier test is an example of the discontinuous solvent extraction method and does not require removal of moisture from the sample. It can be applied to both liquid and solid samples. The Mojonnier flasks are used not only for the Mojonnier and Roese-Gottlieb methods, but also to do the hydrolysis

(acid, alkaline, or combination) prior to fat extraction and GC analysis to determine fat content and fatty acid profile.

18.5.2 Chloroform–Methanol Procedure

The combination of chloroform and methanol has been used commonly to extract lipids. The "Folch extraction" applied to small samples, and the "Bligh and Dyer extraction" applied to large samples of high moisture content, both utilize this combination of solvents to recover lipids from foods. The Bligh and Dyer procedure is a modification of the Folch extraction, designed for more efficient solvent usage for low-fat samples. The Christie modification of these former methods replaced water with 0.88% potassium chloride aqueous solution to create two phases.

In both the modified Folch extraction and Bligh and Dyer procedure, food samples are mixed/homogenized in a chloroform–methanol solution, and the homogenized mixture is filtered into a collection tube. A 0.88% potassium chloride aqueous solution is added to the chloroform–methanol mixture to break into two phases: the aqueous phase (top) and the chloroform phase containing the lipid (bottom). The phases are further separated in a separatory funnel or by centrifugation. After evaporation of the chloroform, the fat can be quantitated by weight. For consistent results, the procedures must be followed carefully, including the ratio of chloroform and methanol. A cautionary note is that chloroform and methanol are highly toxic, so the extraction procedure must be done in well-ventilated areas.

18.6 Total Fat by GC for Nutrition Labeling

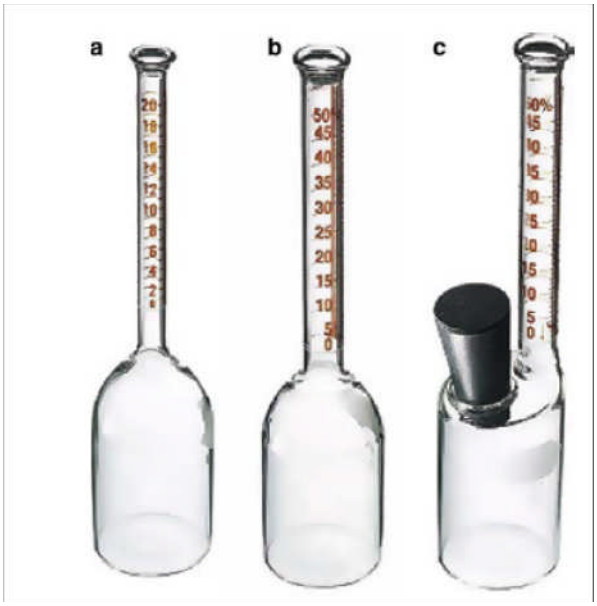
Fat and fatty acids are extracted from food by hydrolytic methods (acidic hydrolysis for most products, alkaline hydrolysis for dairy products, and combination for cheese). Pyrogallic acid is added to minimize oxidative degradation of fatty acids during analysis. Triglyceride, triundecanoin (C11:0), is added as internal standard. Fat is extracted into ether, then methylated to fatty acid methyl esters (FAMEs) using BF3 (boron trifluoride) in methanol.

FAMEs are quantitatively measured by capillary gas chromatography (GC) against C11:0 internal standard. Total fat is calculated as sum of individual fatty acids expressed as triglyceride equivalents. Saturated and monounsaturated fats are calculated as sum of respective fatty acids. Monounsaturated fat includes only *cis* form."

18.7 Nonsolvent Wet Extraction Method

18.7.1 Babcock Method for Milk Fat

In the Babcock method, H_2SO_4 is added to a known amount of milk in the Babcock bottle. The sulfuric acid digests protein, generates heat, and releases the fat. Centrifugation and hot water addition isolate fat for quantification in the graduated portion of the test bottle. The fat is measured volumetrically, but the result is expressed as percent fat by weight.



Babcock milk test bottles for milk

a. cream b. cheese c. testing

18.8 Instrumental Methods

Instrumental methods offer numerous attractive features compared with the previously described extraction methods. In general, they are rapid, nondestructive, and require minimal sample preparation and chemical consumption. However, the equipment can be expensive and measurements often require the establishment of calibration curves specific to various compositions.

18.8.1 Infrared Method

The infrared (IR) method is based on absorption of IR energy by fat at a wavelength of 5.73 µm. The more the energy absorption at 5.73 µm, the higher is the fat content of the sample . Mid-IR spectroscopy is used in Infrared Milk Analyzers to determine milk fat content. Near-infrared (NIR) spectroscopy has been used to measure the fat content of commodities such as meats, cereals, and oilseeds in the laboratory and is being adapted for on-line measurement.

18.8.2 Specific Gravity (Foss-Let Method)

Fat content by the Foss-Let method (Foss North America, Eden Prairie, MN) is determined as a function of the specific gravity of a sample solvent extract. A sample of known weight is extracted for 1.5–2 min in a vibration-reaction chamber with perchloroethylene. The extract is filtered, and using a thermostatically controlled device with digital readout, its specific gravity is determined. The reading can then be converted to oil or fat percentage using a conversion chart.

18.8.3 Nuclear Magnetic Resonance

Nuclear magnetic resonance (NMR) can be used to measure lipids in food materials in a nondestructive way. Total fat content can be measured using low-resolution pulsed NMR. NMR analysis is a very rapid and accurate method, and while the principles of NMR are relatively complex, the use of NMR can be quite simple, especially due to the high degree of automation and computer control.

18.9 Summary

Lipids are generally defined by their solubility characteristics rather than by some common structural feature. Lipids in foods can be classified as simple, compound, or derived lipids. The lipid content of foods varies widely, but quantitation is important because of regulatory requirements, nutritive value, and functional properties. There is no single standard method for the determination of fats in different foods.

The validity of any fat analysis depends on proper sampling and preservation of the sample prior to analysis. Predrying of the sample, particle size reduction, and acid hydrolysis prior to analysis also may be necessary. The total lipid content of foods is commonly determined by organic solvent extraction methods, which can be classified as continuous (e.g., Goldfish), semicontinuous (e.g., Soxhlet), discontinuous (e.g., Mojonnier, Folch), or by GC analysis for nutrition labeling. Nonsolvent wet extraction methods, such as the Babcock or Gerber, are commonly used for certain types of food products. Instrumental methods, such as NMR, infrared, and Foss-Let, are also available for fat determination of specific foods. These methods are rapid and so may be useful for quality control but generally require correlation to a standard solvent extraction method.

18.10 Self Assessment Questions

1. What are the solvent extraction methods for fat analysis?
2. Explain about the semicontinous solvent extraction methods
3. What is chloroform-methonol procedure
4. Explain about various instrumental methods used in analysis of fats.

18.11 Reference Books:

1. S. Suzanne Nielsen, Food analysis, 4th edition, 2010
2. Upadhyay and Upadhyay, Biophysical chemistry, 2006
3. Clifton.E.Meloan, food analysis 3rd edition (theory and practice)

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LESSON - 19

PROTEIN ANALYSIS

19.0 Objective

After going through this lesson you will learn:

- The importance of protein analysis
- Various methods used in protein analysis

Structure

19.1 Introduction

Proteins are an abundant component in all cells, and almost all except storage proteins are important for biological functions and cell structure. Food proteins are very complex. They are composed of elements including hydrogen, x and amino acids are the building blocks of proteins; the amino acid residues in a protein are linked by peptide bonds. Nitrogen is the most distinguishing element present in proteins. However, nitrogen content in various food proteins ranges from 13.4

to 19.1% (1) due to the variation in the specific amino acid composition of proteins. Generally, proteins rich in basic amino acids contain more nitrogen.

Proteins can be classified by their composition, structure, biological function, or solubility properties. For example, simple proteins contain only amino acids upon hydrolysis, but conjugated proteins also contain non-amino-acid components. Proteins have unique conformations that could be altered by denaturants such as heat, acid, alkali, 8*M* urea, 6*M* guanidine-HCl, organic solvents, and detergents. The solubility as well as functional properties of proteins could be altered by denaturants. The analysis of proteins is complicated by the fact that some food components possess similar physicochemical properties. Nonprotein nitrogen could come from free amino acids, small peptides, nucleic acids, phospholipids, amino sugars, porphyrin, and some vitamins, alkaloids, uric acid, urea, and ammonium ions. Therefore, the total organic nitrogen in foods would represent nitrogen primarily from proteins and to a lesser extent from all organic nitrogen-containing nonprotein substances. Depending upon methodology, other major food components, including lipids and carbohydrates, may interfere physically with analysis of food proteins.

Numerous methods have been developed to measure protein content. The basic principles of these methods include the determinations of nitrogen, peptide bonds, aromatic amino acids, dye-binding capacity, ultraviolet absorptivity of proteins, and light scattering properties. In addition to factors such as sensitivity, accuracy, precision, speed, and cost of analysis, what is actually being measured must be considered in the selection of an appropriate method for a particular application.

19.2 Importance of Analysis

Protein analysis is important for:

- Nutrition labeling
- Pricing: The cost of certain commodities is based on the protein content as measured by nitrogen content (e.g., cereal grains; milk for making certain dairy products, e.g., cheese).
- Functional property investigation: Proteins in various types of food have unique food functional properties: for example, gliadin and glutenins in wheat flour for breadmaking, casein in milk for coagulation into cheese products, and egg albumen for foaming.
- Biological activity determination: Some proteins, including enzymes or enzyme inhibitors, are relevant to food science and nutrition: for instance, the proteolytic enzymes in the tenderization of meats, pectinases in the ripening of fruits, and trypsin inhibitors in legume seeds are proteins. To compare between samples, enzymes activity often is expressed in terms of specific activity, meaning units of enzyme activity per mg of protein.

Protein analysis is required to know:

- Total protein content
- Content of a particular protein in a mixture
- Protein content during isolation and purification of a protein
- Non protein nitrogen
- Amino acid composition
- Nutritive value of a protein

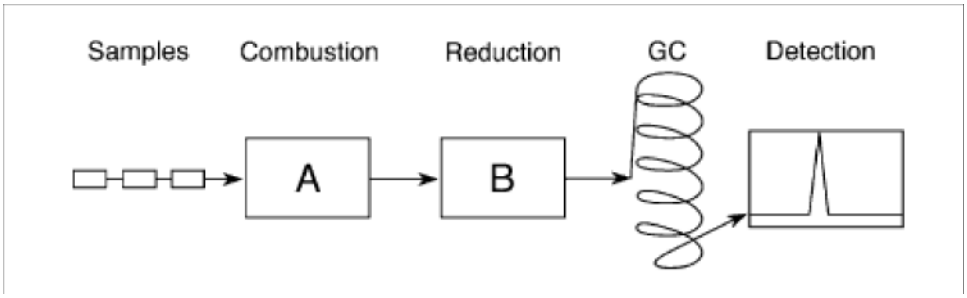
19.3 Methods

19.3.1 Kjeldahl Method

In the Kjeldahl procedure, proteins and other organic food components in a sample are digested with sulfuric acid in the presence of catalysts. The total organic nitrogen is converted to ammonium sulfate. The digest is neutralized with alkali and distilled into a boric acid solution. The borate anions formed are titrated with standardized acid, which is converted to nitrogen in the sample. The result of the analysis represents the crude protein content of the food since nitrogen also comes from nonprotein components.

19.3.2 Dumas (Nitrogen Combustion) Method

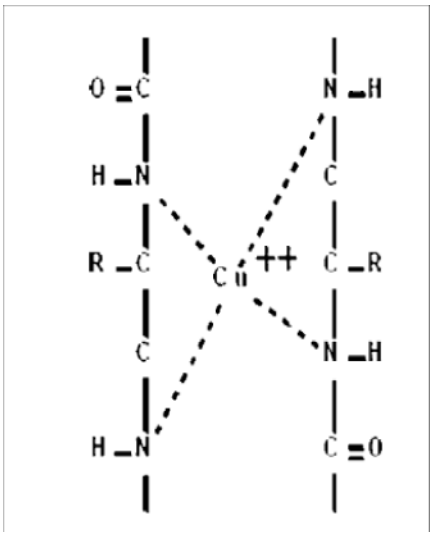
Samples are combusted at high temperatures (700–1000^{0}C) with a flow of pure oxygen. All carbon in the sample is converted to carbon dioxide during the flash combustion. Nitrogen-containing components produced include N2 and nitrogen oxides. The nitrogen oxides are reduced to nitrogen in a copper reduction column at a high temperature (600^{0}C). The total nitrogen (including inorganic fraction, i.e., including nitrate and nitrite) released is carried by pure helium and quantitated by gas chromatography using a thermal conductivity detector (TCD) Ultra-high purity acetanilide and EDTA (ethylenediamine tetraacetate) may be used as the standards for the calibration of the nitrogen analyzer. The nitrogen determined is converted to protein content in the sample using a protein conversion factor.



General components of a Dumas nitrogen analyzer

19.3.3 Infrared Spectroscopy

Infrared spectroscopy measures the absorption of radiation (near- or mid-infrared regions) by molecules in food or other substances. Different functional groups in a food absorb different frequencies of radiation. For proteins and peptides, various mid-infrared bands (6.47 nm) and near-infrared (NIR) bands (e.g., 3300–3500 nm; 2080–2220 nm; 1560–1670 nm) characteristic of the peptide bond can be used to estimate the protein content of a food. By irradiating a sample with a wavelength of infrared light specific for the constituent to be measured, it is possible to predict the concentration of that constituent by measuring the energy that is reflected or transmitted by the sample (which is inversely proportional to the energy absorbed).



Reaction of peptide bonds with cupric ions

19.3.4 Biuret Method

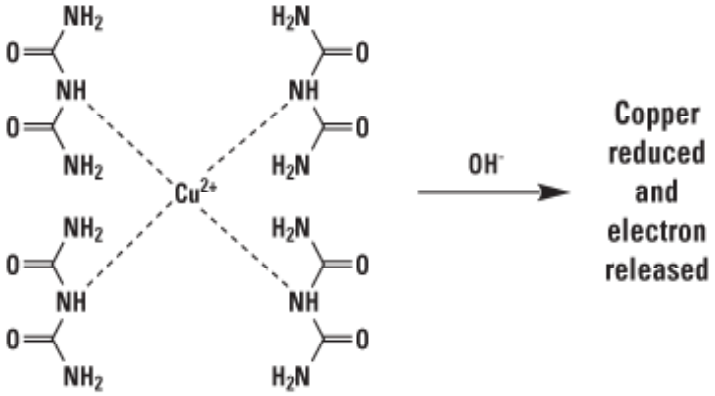
A violet-purplish color is produced when cupric ions are complexed with peptide bonds (substances containing at least two peptide bonds, i.e., biuret, large peptides, and all proteins) under alkaline conditions. The absorbance of the color produced is read at 540 nm. The color intensity (absorbance) is proportional to the protein content of the sample.

Applications

The biuret method has been used to determine proteins in cereal, meat, soybean proteins, and as a qualitative test for animal feed. The biuret method also can be used to measure the protein content of isolated proteins.

Advantages of this method are:

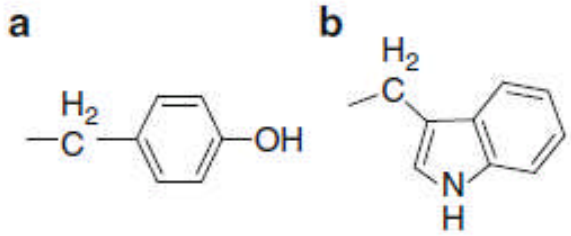
- Less expensive than the Kjeldahl method;
- Rapid (can be completed in less than 30 min);
- Simplest method for analysis of proteins.
- Color deviations are encountered less frequently than with Lowry, ultraviolet (UV) absorption, or turbidimetric methods
- Very few substances other than proteins in foods interfere with the biuret reaction.
- Does not detect nitrogen from nonpeptide or nonprotein sources.



Biuret Reaction

19.3.5 Lowry Method

The Lowry method combines the biuret reaction with the reduction of the Folin–Ciocalteau phenol reagent (phosphomolybdic-phosphotungstic acid) by tyrosine and tryptophan residues in the proteins. The bluish color developed is read at 750nm (high sensitivity for low protein concentration) or ¦



Side chains of amino acids: a. Tyrosin b. Tryptophan

Applications

Because of its simplicity and sensitivity, the Lowry method has been widely used in protein biochemistry. However, it has not been widely used to determine proteins in food systems without first extracting the proteins from the food mixture.

Advantages of this method are:

- Very sensitive
- 50–100 times more sensitive than biuretmethod
- 10–20 times more sensitive than 280-nm UV absorption method Similar sensitivity as Nesslerization; Less affected by turbidity of the sample.
- More specific than most other methods.
- Relatively simple; can be done in 1–1.5 h.

19.3.6 Dye-Binding Methods

19.3.6.1 Anionic Dye-Binding Method

The protein-containing sample is mixed with a known excess amount of anionic dye in a buffered solution. Proteins bind the dye to form an insoluble complex. The unbound soluble dye is measured after equilibration of the reaction and the removal of insoluble complex by centrifugation or filtration.

Protein + excess dye → Protein "dye insoluble complex + unbound soluble dye

The anionic sulfonic acid dye, including acid orange 12, orange G, and Amido Black 10B, binds cationic groups of the basic amino acid residues (imidazole of histidine, guanidine of arginine, and ε-amino group of lysine) and the free amino terminal group of the protein. The amount of the unbound dye is inversely related to the protein content of the sample.

Advantages of this method are:

- Rapid (15 min or less), inexpensive, and relatively accurate for analyzing protein content in food commodities.
- May be used to estimate the changes in available lysine content of cereal products during processing since the dye does not bind altered, unavailable lysine. Since lysine is the limiting amino acid in cereal products, the available lysine content represents protein nutritive value of the cereal products.
- No corrosive reagents.
- Does not measure nonprotein nitrogen.
- More precise than the Kjeldahl method.

19.3.6.2 Bradford Dye-Binding Method

When Coomassie Brilliant Blue G-250 binds to protein, the dye changes color from reddish to bluish, and the absorption maximum of the dye is shifted from 465 to 595 nm. The change in the absorbance at 595nm is proportional to the protein concentration of the sample. Like other dye-binding methods, the Bradford relies on the amphoteric nature of proteins. When the protein containing solution is acidified to a pH less than the isoelectric point of the protein(s) of interest, the dye added binds electro-statically. Binding efficiency is enhanced by hydrophobic interaction of the dye molecule with the polypeptide backbone adjoining positively charged residues in the protein. In the case of the Bradford method, the dye bound to protein has a change in absorbance spectrum relative to the unbound dye.

Advantages of this method are:

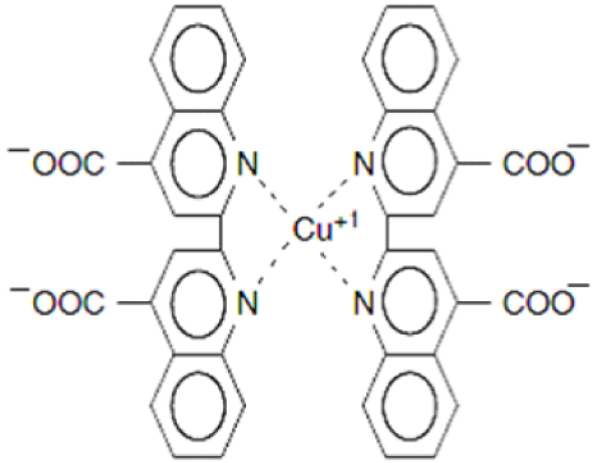
- Rapid; reaction can be completed in 2 min
- Reproducible
- Sensitive; several fold more sensitive than the Lowry method
- No interference from ammonium sulfate, polyphenols, carbohydrates such as sucrose, or cations such as K+, Na+, and Mg^{2+}
- Measures protein or peptides with molecular mass approximately equal to or greater than 4000 Da

Applications

The Bradford method has been used successfully to determine protein content in worts and beer products and in potato tubers. This procedure has been improved to measure microgram quantities of proteins. Due to its rapidity, sensitivity, and fewer interferences than the Lowry method, the Bradford method has been used widely for the analysis of low concentrations of proteins and enzymes in their purification and characterizations.

19.3.7 Bicinchoninic Acid Method

Proteins and peptides (as short as dipeptides) reduce cupric ions to cuprous ions under alkaline conditions, which is similar in principle to that of the biuret reaction. The cuprous ion then reacts with the apple-greenish bicinchoninic acid (BCA) reagent to form a purplish complex (one cuprous ion is chelated by two BCA molecules). The color measured at 562nm is near linearly proportional to protein concentration over a wide range of concentration from micrograms up to 2mg/ml. Peptide bonds and four amino acids (cysteine, cystine, tryptophan, and tyrosine) contribute to the color formation with BCA. The BCA method has been used in protein isolation and purification.



Protein reaction with cupric ions under alkaline conditions to form cuprous ions, which react with bicinchninic acid to form purple colour, measure at 562 nm

Advantages of this method are:

- One-step mixing is easier than in the Lowry method.
- The reagent is more stable than for the Lowry reagent.
- Nonionic detergent and buffer salts do not interfere with the reaction.
- Medium concentrations of denaturing reagents (4*M* guanidine-HCl or 3*M* urea) do not interfere.

19.3.8 Ultraviolet 280nm Absorption Method

Proteins show strong absorption in the region at ultraviolet (UV) 280nm, primarily due to tryptophan and tyrosine residues in the proteins. Because the content of tryptophan and tyrosine in proteins from each food source is fairly constant, the absorbance at 280nm could be used to estimate the concentration of proteins, using Beer's law. Since each protein has a unique aromatic amino acid composition, the extinction coefficient (*E*280) or molar absorptivity (*Em*) must be determined for individual proteins for protein content estimation.

Advantages of this method are:

- Rapid and relatively sensitive; At 280 nm, 100 µg or more protein is required; several times more sensitive than the biuret method.
- No interference from ammonium sulfate and other buffer salts.
- Nondestructive; samples can be used for other analyses after protein determination; used very widely in post column detection of proteins.

19.4 Summary

Methods based on the unique characteristics of proteins and amino acids have been described to determine the protein content of foods. The Kjeldahl and Dumas methods measure nitrogen. Infrared spectroscopy is based on absorption of a wavelength of infrared radiation specific for the peptide bond. Copper–peptide bond interactions contribute to the analysis by the biuret and Lowry methods. Amino acids are involved in the Lowry, dye-binding, and UV 280nm methods. The BCA method utilizes the reducing power of proteins in an alkaline solution. The various methods differ in their speed and sensitivity.

19.5 Self Assessment Questions

1. Write short note on the following
   a. Kjeldahl method
   b. Biuret method
   c. Bicinconinic acid method
   d. Infrared spectoscopy
2. What are the dye binding methods for protein analysis?

19.6 Reference Books:

1. S. Suzanne Nielsen, Food analysis, 4th edition, 2010
2. Upadhyay and Upadhyay, Biophysical chemistry, 2006
3. Clifton.E.Meloan, food analysis 3rd edition (theory and practice)

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