FOOD CHEMISTRY AND ANALYSIS

M.Sc. FOOD AND NUTRITION SCIENCE

SEMESTER-I, PAPER-II

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M.Sc. FOOD AND NUTRITION SCIENCE: FOOD CHEMISTRY AND ANALYSIS

First Edition : 2025

No. of Copies :

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Published by:

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Printed at:

FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

Prof. K. Gangadhara Rao
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M.Sc. FOOD AND NUTRITION SCIENCE
SEMESTER-I, PAPER-II
102FN24-FOOD CHEMISTRY AND ANALYSIS

SYLLABUS

Course Objectives: To enable the students to

1) Acquire knowledge on chemical composition of different foods.
2) Understand the physical, chemical, and functional properties of foods.
3) Know the principles and working applications of different analytical techniques associated with food.
4) Perform skills in qualitative and quantitative estimation of nutrients in different foods.

THEORY

Unit-I:

Water Chemistry and Dispersed Systems

- Water Chemistry-Structure of Water, Free, Bound and Entrapped Water.
- Water Activity and Relative Vapour Pressure-Definition and measurement, factors affecting water activity, Moisture sorption isotherms, Hysteresis and Moisture Determination.
- Dispersions-Food as dispersed systems, Liquid Dispersions.
- Colloids-Definition, Characteristics of Colloids, Gels, Emulsions, Foams.

Unit-2:

Starch Chemistry:

- Types of starches, chemical structure of starch, properties of different starches, method of extraction of starch, determination of reducing sugars and non reducing sugars and crude fibre.

Lipid Chemistry:

- Lipids-Nomenclature, classification-Milk fats, Animal fats, Vegetable fats.
- Physical Properties-Crystallization, Plasticity
- Chemical Properties Thermal decomposition, Chemistry of Frying, Hydrogenation, Inter esterification, Rancidity of fats.
- Fats-Analysis of solid and liquid fats, Rancidity.

Unit-3:

Protein Chemistry:

- Nature and Types of Proteins-Plant foods, Egg, Milk and fleshy foods, properties of different proteins.
- Proteins Electrophoresis, Micro-Kjeldahl method.

Unit-4:

Fruits and Vegetables:

- Post Harvesting Changes-Chemistry-Composition of Fruits and Vegetables. Plant Tissues and Relationship with Texture.

Plant Pigments:

- Water Insoluble Plastid Pigments- Chlorophyll and Carotenoids.
- Chemical Structure.
- Water Soluble Pigments-Anthocyanins, Anthoxanthins, Flavones and Tannins.

Food Enzymes:

- Types of Enzymes in Foods and their Importance to Food Quality.
- Methods of Determination of Total Ash.
- Vitamins and Minerals-Ca, Phosphorus, Iron, Vitamin A, Beta Carotene, Riboflavin and Vitamin C.

Unit-5:

Instrumentation:

- Basic Principles and Applications of Spectroscopy-UV, UV-visible, AAS, AES, Electromagnetic Resonance.
- Chromatography-Principles and Applications of Chromatography-HPLC, GC/MS and LC/MS.

References:

1) Berk.Z., Introduction to Bio-chemistry of Foods, Dept. of Food Engineering and Biotechnology, Israel Institute of Technology, Amsterdam, New York.
2) Clipton. E. Meloan, Food Analysis 3rd Edition (Theory & Practice).
3) David and Robinson, Bio-Chemistry and Nutritional Value.
4) Dennis. D, Muller, Food Chemistry, A Laboratory Manual by Inter Science Publication, John Willey & Sons Inc.
5) W.S. Wong, Mechanism and Theory of Food Chemistry, CBS Publishers and Distributors 1996.
6) Seemayadav, Food Chemistry, Publication of Anmolpvt., Ltd., 1997.
7) Owen R. Food Chemistry 2nd edition.

Course Outcomes: After completion of this course, students will be able to:

CO1: Develop an understanding of different forms of water and water activity

CO2: Acquire knowledge on chemical nature and analytical techniques of starch and lipids.

CO3: Analysis and identification of protein molecules in plant and animal food stuffs.

CO4: Identification of post harvesting changes in fruits and vegetables. Analytical techniques of micronutrients in fruits and vegetables.

CO5: Provide awareness about the principles, methods and applications of spectroscopy and chromatography techniques.

(102FN24)

M.Sc. DEGREE EXAMINATION, MODEL QUESTION PAPER
FIRST SEMESTER
FOOD CHEMISTRY AND ANALYSIS

Time: Three hours **Maximum: 70 marks**

Answer ONE Question From Each Unit **5 × 14 = 70M**
Each Question Carries 14 Marks.

UNIT-I

1 Explain about Water activity and Relative vapour pressure?

OR

2 Discuss about the colloids?

UNIT-II

3 What are the different methods of extraction of starch? Explain it.

OR

4 Enumerate the chemical properties of lipids?

UNIT-III

5 Write about the nature and types of proteins?

OR

6 Discuss in detail about Micro-Kjel dhal method?

UNIT-IV

7 What are the water insoluble plastid pigments?

OR

8 Explain about types of enzymes in foods and their importance of food quality?

UNIT-V

9 Write the basic principles of spectroscopy?

OR

10 Discuss in detail about chromatography?

CONTENTS

LESSON-1

WATER CHEMISTRY

1.0 OBJECTIVES:

- To know about structure and types of water
- To study about water activity and relative vapour pressure
- To understand moisture sorption isotherms

STRUCTURE:

1.1 INTRODUCTION:

Water is the major constituent of life. It is the widely distributed compound as liquid, solid, gas and is the crucial component in food. It acts as a medium for all the cell reactions and maintains electrolyte balance of the body. It regulates the body temperature and acts as both reactant and product. Water is responsible for all the changes that take place during cooking of different foods and is also known as food solvent. Water dissolves flavours as well as acts as dispersion medium. Excess water in food leads to spoilage and it should be as low as possible to enhance stability.

Water has certain physical properties like melting point, boiling point, surface tension, specific heat and dielectric constants. Water exhibits maximum density at 4 °C. Its Freezing point is 0°C. Ice has more thermal conductivity than water i.e., almost 4 times at same temperatures. These all properties are very unusual in other liquids, making it a good dispersion medium for a variety of foods.

1.2 PHYSICAL PROPERTIES OF WATER:

Table 1.1: Properties of Water

Property	Value
Molecular Weight	18.0153
Melting Point(at 101.3kPa)	0.00◦C
Boiling Point(at 101.3kPa)	100.00◦C
Critical temperature	373.99◦C
Critical pressure	22.064Mpa
Triplepoint temperature	0.01◦C
Triplepoint pressure	611.73Pa
Density (g/cm3)	0.99984
Vapour Pressure (kPa)	0.6113
Heat Capacity (J/g/K)	4.2176
Thermal Conductivity (W/mK	0.561
Thermal Diffusivity (m2/s)	1.3
Compressibility (Pa-1)	4.9
Permittivity	87.9

(Source- Food Chemistry by Owen R Fennema)

1.3 STRUCTURE OF WATER:

Water molecule is formed by two hydrogen atoms forming covalent bonds with an oxygen atom. Molecular formula for water is H2O. Bond angle is 104.5◦C. Water is an electrically neutral molecule, because of the arrangement of the atoms. Oxygen atoms on one side are slightly electro negative and hydrogen atoms are positive. Due to the polar nature of water molecules, each hydrogen atom is attracted to the oxygen atom of another molecule and the oxygen atom to the hydrogen atom. This leads to multiple hydrogen bonding on a three dimensional basis.

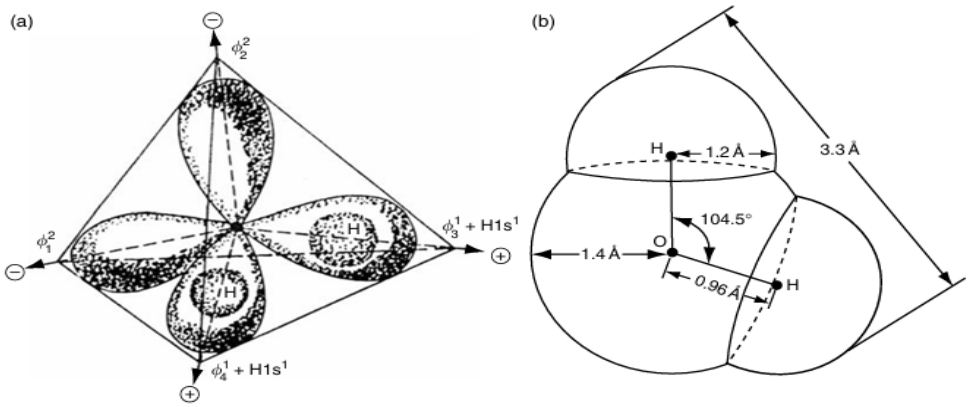

Figure 1.1: Schematic model of Single HOH molecule

(a) Possible sp3 configuration and (b) van der Waals radii for a HOH molecule in vapor state.

(Source- Food Chemistry by Owen R Fennema)

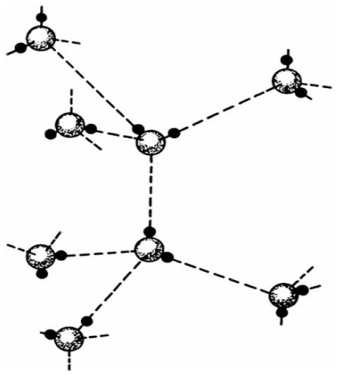

Figure 1.2: Hydrogen Bonding of Water Molecules in a Tetrahedral Configuration.

(Source- Food Chemistry by Owen R Fennema)

In the above diagram, open circles are oxygen atoms, closed circles are hydrogen atoms. Hydrogen bonds are represented by dashed lines. In line, Water is also termed as an open liquid, having a density only 60% of that expected in liquids. This is due to the close pack of molecules. This is due to partial retention of open, hydrogen - bonded tetrahedral arrangement of ice.

Theories of Water Structure:

Theories of water structure are classified into two major classes. They are given below

1) Homogeneous models
2) Mixture models
3) Continuum models (homogenous or uniformist models)

According to homogenous models, intermolecular hydrogen bonds are distributed throughout the water, so that each water molecule has essentially the same environment. Mixture model states that intermolecular hydrogen bonds are concentrated, at any given moment, in localized multimolecular clumps or clusters of water molecules. The Continuum model suggests that intermolecular hydrogen bonds are distributed uniformly throughout the

sample, and that many bonds in ice become distorted when ice is melted. However, out of all, Mixture models are mostly seen in water systems.

Every model permits each and every water molecule to alter bond arrangements frequently and also terminates hydrogen bond in exchange for a new one. In the whole process, constant temperature, constant hydrogen bonding and structure are maintained.

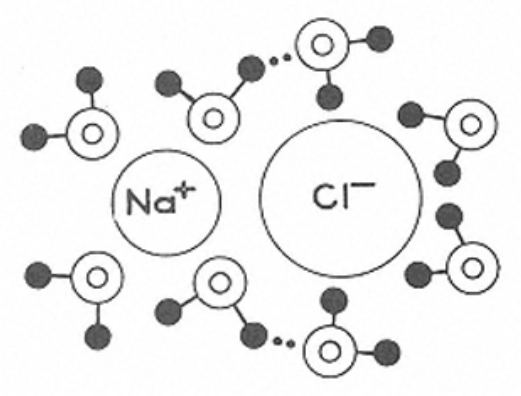

Figure 1.3: Water Molecules in Relation with Sodium Chloride

(Source- Food Chemistry by Owen R Fennema)

1.4 TYPES OF WATER:

All natural foods are composed of nearly 70% water of their weight. Based on molecular interactions, water in foods is divided into three categories.

1) Free water
2) Bound water
3) Entrapped water

Water which can be extracted easily from foods by cutting, squeezing or pressing is known as free water. Water that is difficult to remove is termed as bound water. Entrapped water is seen in capillaries or cells and is immobile in nature. If we give any external pressure, it flows freely. Bound water can be frozen only at low temperatures unlike free water. Most of it binds tightly with solutes like protein and has density more than water. Based on the functionality, it is divided into 3 types.

1) Constitutional
2) Vicinal
3) Multilayer

Constitutional water forms the integral part of a non aqueous constituent accounting for 0.03% of total water. Vicinal water has specific hydrophilic sites of non aqueous constituents to form a monolayer constituting 0.1 to 0.9%. Multilayer water contains several layers around hydrophilic groups and covers 1 to 5% of total water.

1.5 WATER ACTIVITY AND RELATIVE VAPOUR PRESSURE:

The ratio between vapour pressure in food and vapour pressure of distilled water, in a balanced environment with the surrounding media is known as water activity. All the conditions should be identical in nature. A water activity of 0.70 means the vapor pressure is 70 percent of that of pure water. The water activity and temperature are directly proportional to each other. Moisture of the product is measured in terms of equilibrium relative humidity (%). Since water activity is a ratio, it has no units.

Table 1.2: Minimum Water Activity for the Growth of Microbes

Aw Range	Microorganisms Inhibited by Lowest aw in this Range
1.00–0.95	Pseudomonas, Escherichia, Proteus, Shigella, Klebsiella, Bacillus, Clostridium
0.95–0.91	Salmonella, Vibrio parahaemolyticus, Clostridium botulinum A, B, Listeria, monocytogenes, Bacillus cereus
0.91–0.87	Staphylococcus aureus (aerobic), many yeasts (Candida, Torulopsis, Hansenula), Micrococcus)
0.87–0.80	Most molds (mycotoxigenic penicillia), Staphylococcus aureus, most Saccharomyces (bacilii) spp., Debaryomyces
0.80–0.75	Most halophilic bacteria, mycotoxigenic aspergilli
0.75–0.65	Xerophilic molds (Aspergillus chevalieri, A. candidus, Wallemia sebi), Saccharomyces, bisporus
0.65–0.61	Osmophilic yeasts (Saccharomyces rouxii), a few molds (Aspergillus echinulatus, Monascus bisporus)
< 0.61	No microbial proliferation

Source: *Beuchat, L.R. (1981) Cereal Foods World 26: 345–349*

Water activity above 0.95, supports the growth of bacteria, yeasts, fungi and mold. The amount of available moisture can be reduced through several techniques, to inhibit the growth of microbes. Water activity is a measurement of water content. The below formula gives the water activity concept in relation with vapour pressure

$$aw = P / P\,0$$

Where, P = Vapor pressure exerted by water present in food

P0 = Vapor pressure of pure water

Foods with higher water activity give way for many microbes to grow. Bacteria require 0.91, fungi 0.7. Since water migrates from high water active areas to areas of low water activity, when foods are exposed to a humid environment, they absorb water and become hygroscopic.

Table 1.3: Minimum Water Activity for the Growth of Microbes

Food Product	Approximate aw Value
Fresh foods: Milk, vegetables, fruits, and meats	0.97–0.99
Canned products	0.97–0.98
Yogurt	0.98
Tomato paste (double)	0.98
Leberwurst	0.97
Mozzarella cheese	0.97
Processed cheese	0.97
White bread (sliced)	0.96
Pâté de foie gras	0.95–0.96
Mortadella (Italian)	0.95
Mayonnaise	0.95
Margarine	0.94
Salted olives	0.93–0.95
Fresh pasta (MA packaged)	0.92–0.94
Tomato ketchup	0.93
Soybean sauce	0.92
French dressing	0.92
Parmesan cheese	0.91
Salami	0.9
Intermediate-moisture category food	0.88
Chocolate syrup	0.86

Source: *Water Activity in Foods: Fundamentals and Applications, Second Edition. R Labuza, 2020*

Measurement of Water Activity:

Water activity is measured using hygrometers, which typically employ sensors to detect the equilibrium relative humidity of the air in a sealed chamber containing the food sample. Along with hygrometers, the below listed water activity meters were also used.

1) Capacitance sensors: Measure changes in capacitance as the humidity in the air changes.
2) Chilled-mirror dew point sensors: Measure the temperature at which dew forms on a mirror, which correlates with the air's relative humidity.
3) Resistive electrolytic sensors: Detect changes in the electrical resistance of a salt solution that absorbs moisture.

Procedure: Food Samples can be tested in duplicate. Instruments are set up, where data obtained from humidity generators is decoded. sodium chloride, potassium nitrate, and potassium sulfate are used as salt slushing agents. These salts will give a range of water activity (at 25 C) from 0.759 to 0.968. The results in tests are confirmed by using pressure equilibrium techniques. In these, Microcrystalline cellulose is used as a reference standard.

Factors for Controlling Water Activity:

1) Ingredients of final product
2) Water binding capacity of ingredients
3) ERH percentages
4) Method of determination of ERH
5) Formulation of product
6) Packaging of product
7) Temperature control in Supernatant air
8) Accurate reading of water activity value

1.5.1 Factors Affecting Water Activity:

Water activity in food products depends on following factors:

1) Food Composition: Ingredients such as sugars, salts, and proteins can bind water, lowering the water activity. The presence of solutes reduces the free water available, leading to a lower water activity.
2) Temperature: Water activity increases with temperature because higher temperatures increase the energy of water molecules, making them more likely to enter the vapor phase. This can affect storage conditions and shelf life predictions.
3) Processing Techniques: Methods like drying, freezing, and the addition of humectants (substances that retain moisture) can significantly alter a product's water activity. For instance, drying reduces water activity by removing free water, while freezing immobilizes water in a solid state, lowering aw.

4) Packaging: The type of packaging and its permeability to moisture can influence the water activity over time. Moisture-barrier packaging helps maintain a consistent water activity level by preventing moisture exchange with the environment.

Understanding these factors is vital for controlling water activity in food production and storage, ensuring that products remain safe and stable throughout their shelf life.

1.5.2 Moisture Sorption Isotherms:

Definition: A plot of water content (expressed as mass of water per unit mass of dry material) of a food versus p/p0 at constant temperature is known as a moisture sorption isotherm (MSI)**.**

Moisture Sorption Isotherms will be used in food systems for determining the below functions.

a) Concentration and dehydration processes, because the ease or difficulty of water removal is related to relative vapour pressure

b) Formulation of food mixtures in order to avoid internal moisture transfer

c) Moisture barrier properties for food packaging material

d) Determination of spoilage factor in foods

e) Predicting stability of foods

Various types of isotherms are seen in foods based on sample composition, physical structure, pretreatments, temperature and processing methodology. Adsorption isotherms are seen when water is added to dry samples. Adsorption is also known as resorption. Sigmoidal shapes are seen in most foods, also known as desorption isotherms.

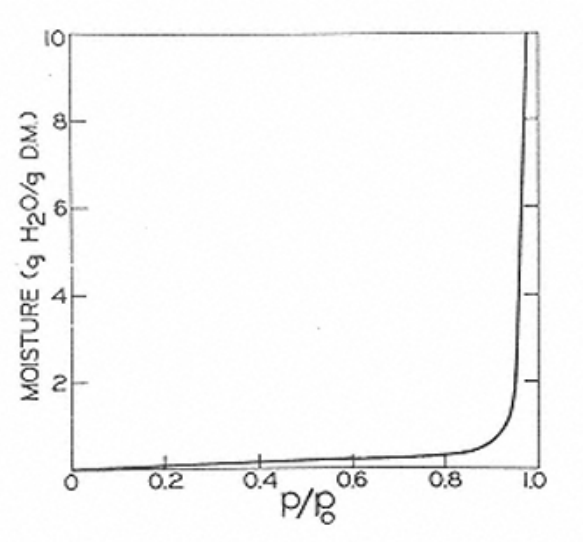

Figure 1.4: Moisture sorption Isotherms depicting wide range of moisture contents

(Source- Food Chemistry by Owen R Fennema)

Foods such as fruits, confections, and coffee extract that contain large amounts of sugar and other small, soluble molecules and are not rich in polymeric materials exhibit a J-type isotherm also. The oldest and best known model is that of Brunauer, Emmett, and Teller. One of the best models is that developed by Guggenheim , Anderson, and DeBoer, and this is referred to as the GAB model.

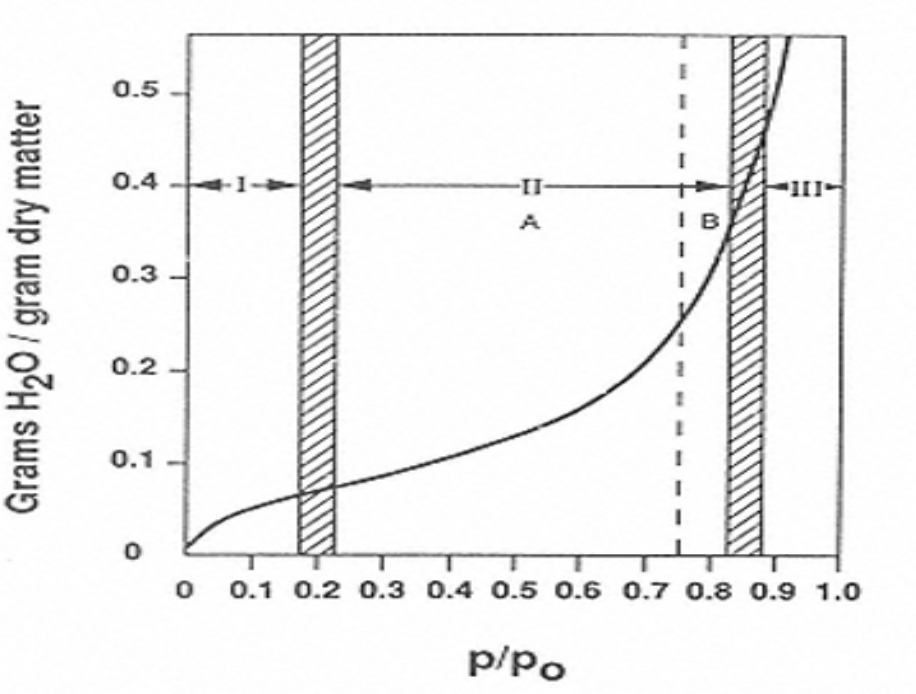

Figure 1.5: **Moisture Isotherm for Low Segments of Food (20C)**

(Source- Food Chemistry by Owen R Fennema)

Moisture sorption isotherms are divided into several zones. If water is added to the sample, it absorbs water and the sample moves from the drier zone to the higher moisture zone. Dry zone is known as Zone-1 and the high moisture zone is known as Zone -III. In all the zones, water properties differ.

Characteristics of Water in Zone-1

1) Water is strongly sorbed
2) Least Mobile water
3) Association of water with accessible polar sites
4) Water -Ion or Water -dipole interactions
5) Water unfreezable at -40°C,
6) Less ability to dissolve solutes
7) Less plasticizing effects on solids
8) Lower amount of water

Characteristics of Water in Zone -II

1) Water in Zone-II appears as first layer sites in food
2) Hydrogen bonding with solute particles and neighbouring water molecules
3) Slightly less mobile than bulk water
4) Unfreezable at 40°C
5) Plasticizing action seen on solutes
6) Lowers glass transition temperature
7) Swelling of solid matrix
8) Acceleration in rate of reactions
9) Constitutes less than 5% water

Characteristics of Water in Zone-III

1) True monolayer hydration is seen
2) Covers macromolecules like protein
3) Lowers glass transition temperatures of macromolecules
4) Equalizes sample temperature
5) Referred as bulk phase water
6) Addition of more water decreases viscosity
7) Increase in molecular mobility
8) Increase in rate of reactions
9) Freezable water
10) Water available as solvent
11) Supports the growth of microbes
12) Water is physically entrapped and flow is impeded

1.5.3 Hysteresis:

Moisture sorption isotherms are generally prepared by addition of water to dry samples, which is known as resorption. It might not be super imposable on an isotherm prepared by addition of water. This lack of super impossibility is known as hysteresis. At any given vapour pressures, the moisture content of the sample will be higher during desorption than during resorption. Hysteresis is seen in glasses of low molecular weight compounds, polymers etc.,

Hysteresis is observed in terms of magnitude of hysteresis, shape of curves, inception and termination points of hysteresis loop. These all depend on certain factors like, nature of food, physical changes in food when water is added or removed, temperature, rate of desorption, degree of water removal during desorption etc., Hysteresis is not detectable at higher temperatures and is evident when temperature is lowered. When temperatures lower, swelling, metastable local domains, chemisorption, phase transitions, capillary phenomena, and the fact that nonequilibrium states become increasingly persistent.

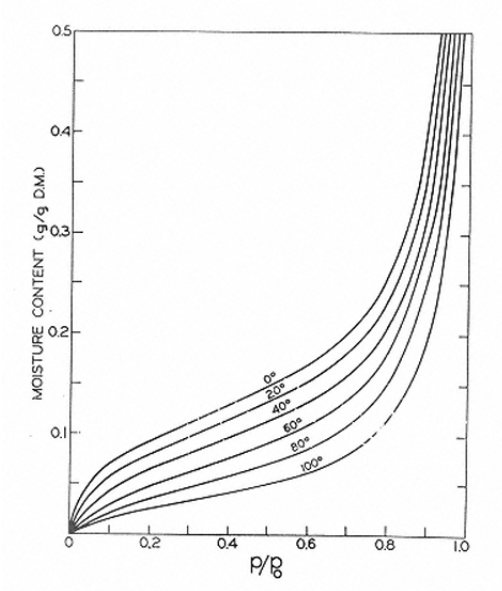

Figure 1.6: Moisture Desorption Isotherms of Potatoes at Various Temperatures

(Source- Food Chemistry by Owen R Fennema)

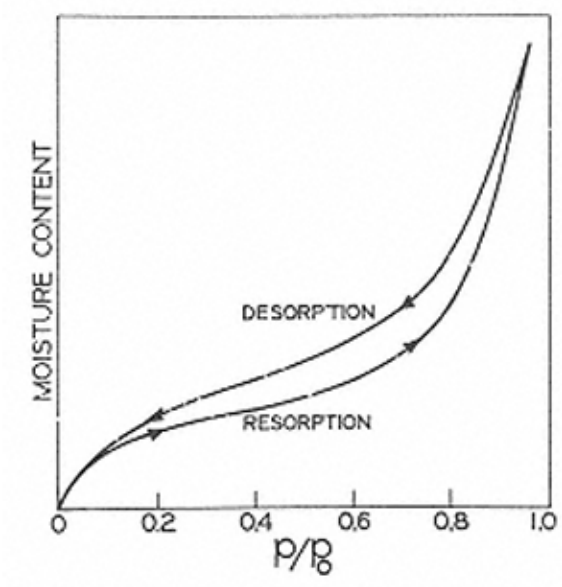

Figure 1.7: Hysteresis of Moisture Sorption Isotherms

(Source- Food Chemistry by Owen R Fennema)

MSIs are highly product specific, that the MSI for a given product can be changed significantly by the manner in which the product is prepared.

1.6 SUMMARY:

Water activity controls the stability of foods. Higher water activity indicates perishability of foods and has to be controlled. Water activity in food can be suppressed by different methods like dehydration, drying, Salt and sugar addition etc., these methods prevent all kinds of food spoilage and maintain food quality. The water activity elaborates the energy state of water present in food, and hence it is also known as solvent which participates in biochemical reactions and growth of microorganisms. Water activity is used to predict the stability and safety of food with respect to microbial growth, rates of deteriorative reactions and chemical or physical properties.

Quality of food depends on concentration on hydrogen ions (pH) and water activity in the food matrix. Water activity, pH, temperature, and other parameters, have a direct impact on the growth of microorganisms, thus water activity and pH are two of the most important parameters. Free water is responsible for the growth of molds, yeasts, and bacteria and even toxin production. This might deteriorate texture, flavor, color, taste, nutritional value of a product, along with stability. Water activity is used to predict the growth of spoilage microbes. Hence water activity is an important parameter to be checked to improve stability of foods.

1.7 TECHNICAL TERMS:

Water Activity, Bound Water, Free water, Equilibrium Moisture Content, Relative Vapour Pressure, BET Monolayer model, Hysteresis, Moisture Sorption Isotherms.

1.8 SELF ASSESSMENT QUESTIONS:

1) Elucidate the structure of water?
2) Write about types of water?
3) Define Water activity. What are the factors affecting water activity?
4) Write about moisture sorption Isotherms?
5) Explain hysteresis phenomenon in foods?

1.9 SUGGESTED READINGS:

1) Owen R Fenemma, Food Chemistry, 4th Revised Edition, 2007.

2) Srinivasa Damodaran, Kirk L Parkin, Owen R Fenemma, Food Chemistry 3rd Revised Edition, 1996.

3) H.D. Belitz, W.Grosch and P.Schieber. Food Chemistry 4th Revised Edition, 2009.

4) Water Activity in Foods, Technical Guidance Document, Food and Drug Administration, 2019.

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Dr. B. Babitha

LESSON-2

MOISTURE ANALYSIS

2.0 OBJECTIVES:

- To know about importance of moisture analysis in foods
- To know about physical and chemical properties of water in foods
- To study about methods of moisture analysis

STRUCTURE:

2.1 INTRODUCTION:

Moisture is one of the important analyses related to food matrices. Moisture defines the amount of water present in the food. Water molecules in food are generally small in nature and transform into different forms based on the environmental changes. The presence of moisture in food makes food fresh but at the same time, excess moisture may cause spoilage. Hence moisture is termed as stability and quality determining factor in most of the foods. Along with water content, water activity is also an important factor to give complete moisture analysis. Water activity measures the total water available to micro organisms for growth.

Importance of Moisture Analysis:

Moisture analysis is one of the fundamental analyses for foods. Before analysing any component, moisture determination should be done. Water can also be termed as moisture in some cases. After removing moisture, dry matter remains and are known as total solids. Moisture along with total solids will determine the shelf life of foods. Moisture analysis is very important for food processing industries for processing products with national and international standards. Not only the total amount of water but the energy states of water also should be estimated in terms of water activity. If water activity is higher than the moisture, deterioration occurs. Always moisture migrates from higher water activity ranges to lower water activity ranges, which leads to instability of food components. Hence, it is necessary to determine the amount of moisture in foods.

2.2 METHODS OF MOISTURE ANALYSIS:

Every food matrix has different moisture levels. In line, they are categorized into perishable and non perishable foods. Based on moisture levels, methods of determination vary. Practicality and accuracy are the two key factors for good analysis. Based on the method, moisture may be analyzed more and sometimes less than actual amount. Hence official methods of analysis have been suggested by national and international forums. There are two types of methods for moisture analysis

1) Direct Analysis
2) Indirect Analysis

In direct analysis, steps like drying, distillation, extraction, weighing, volumetry, titration are included. Indirect methods are based mostly on capacitance, specific gravity, density, freezing point etc.,

2.3 DIRECT METHODS:

2.3.1 Oven Drying Method:

Oven drying method is a direct method for moisture analysis which includes the following steps

1) Sample preparation
2) Heating
3) Determining loss in sample weight
4) Calculation of moisture content

Factors Affecting Oven Drying Method:

1) Type of food Sample
2) Type of oven
3) Conditions in oven

4) Time
5) Temperature

AOAC International approved some ovens as safe and is used worldwide. This is a simple effective method where larger samples are analysed within an hour to 24 hrs. Temperature is the key factor for removing moisture at a higher rate. The Oven method employs Raoult's law, which states that 1 molecule of solute dissolved in 1 Litre of solvent (mostly water), boiling point will be raised by 0.512°C. Throughout the drying process, solute concentration increases, as well as boiling point is elevated.

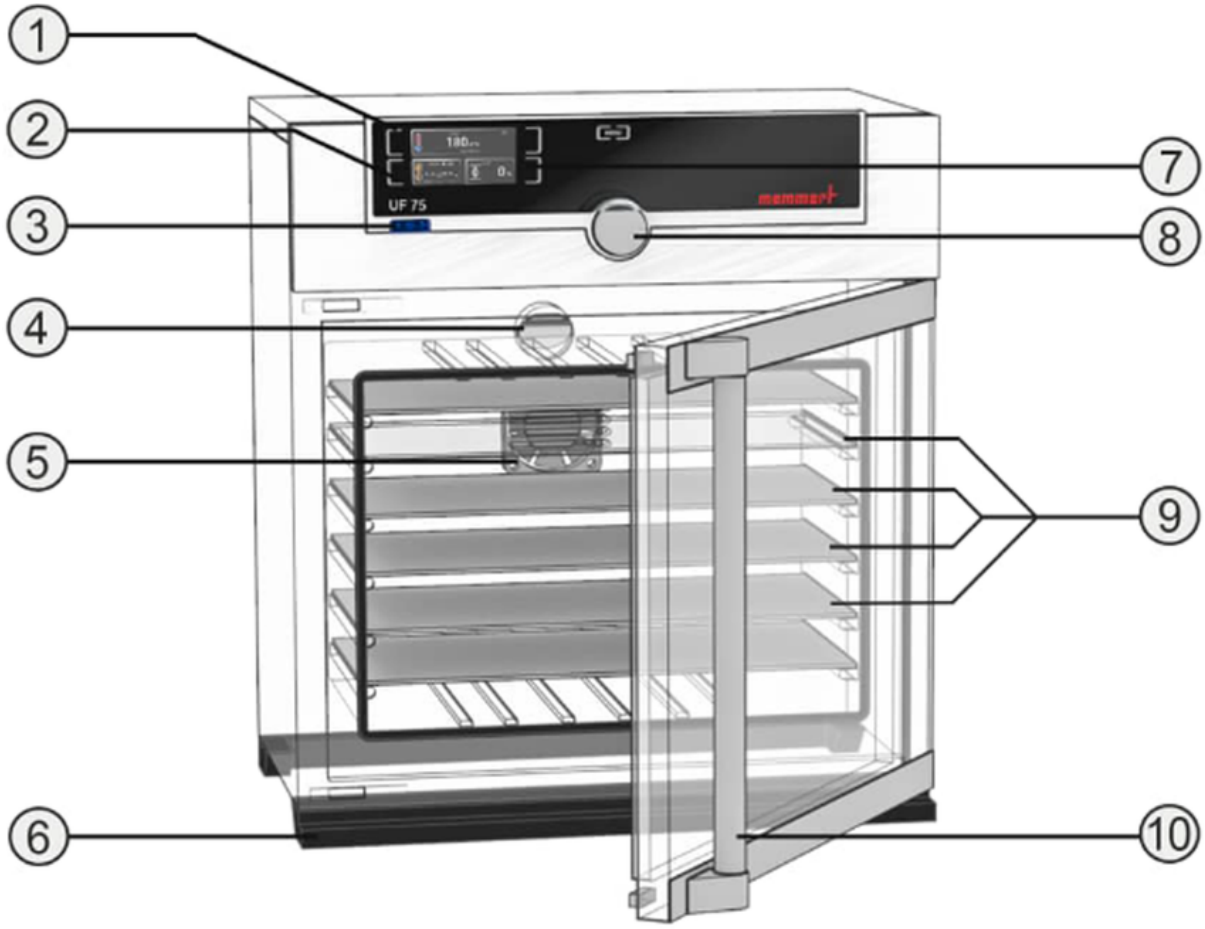


Figure 2.1: Schematic view of the Hot Air Oven

Source: Journal of Food Process Engineering, 2018

1) Temperature display
2) Fan speed display
3) ON/OFF button
4) Air flap
5) Axial fan
6) Base stand
7) Air flap display

8) Control knob (for temperature, fan speed, and air flap levels)
9) Drying trays
10) Oven door

A two stage process of moisture removal is seen. For liquids, pre drying over a steam bath is essential before actual drying is employed. Bread and related crumbs are air dried, ground and oven dried. Drying rate is influenced by particle distribution, size, hygroscopicity along with surface area, which directly influences efficiency of moisture removal. Time and temperature play a major role in the drying phenomenon. High temperature and high time decomposes food more. Controlled decomposition is essential and is critical to obtain. Any physical process should separate all the moisture content without decomposing any one of the constituents that release water.

Sometimes overestimation occurs when carbohydrates decompose faster than other components. Underestimation is also seen, when sucrose is hydrolysed by some chemical reactions. Error in output comes, when loss of volatiles occurs. More moisture will be reported if oxidation of unsaturated fatty acids occurs**.** Temperature should be controlled by the use of an appropriate oven. Based on the extent of variation within the oven, precautions should be taken. For example, Convection oven shows higher temperature variation. Forced draft oven shows least temperature variation. Vacuum oven spreads the temperature more than any other method.

Types of Pans for Oven Drying Methods:

Drying Pans should be approved by AOAC international with 5.5 cm width. Insert cover is mandatory. Metal covers should be avoided, so that slippage does not occur. Usage of disposable pans is also recommended.

Majority of the analytical procedures include vacuum ovens. The information obtained through vacuum ovens is very sensitive and has to follow some instructions as given below

1) Temperature employment depends on the nature of foods. For fruits and high sugar foods, lower temperatures should be used. For grains and other stable products, higher temperatures can be used.
2) Correction factors should be used when dealing with high concentrated volatiles. This helps to avoid loss
3) Pans should be placed directly on metal shelves for proper heat conduction
4) Condensation is very often in samples after evaporation. Care should be taken so that condensed products will not drop the temperature.
5) The drying interval is checked regularly for more reproducability.

2.3.2 Microwave Analyzer:

Microwave analysis, often called microwave drying, is the faster process to check moisture right before packaging the final product. This reduces production costs and ensures consistency.

When using a microwave analyzer, the following necessities should be noted.

1) Uniformity in size
2) Proper distribution of sample to avoid burning
3) Consideration of sample pads
4) Consideration of type of pads (Fibre glass or quartz)
5) Check on Absorption patterns of sample pads

Vacuum microwave oven can also be used for proper estimation. Major advantage is the accurate and faster output. Disadvantage is that only single samples are analyzed.

2.3.3 Infrared Drying:

It involves heat penetration into the sample rather than surface conduction or convection. Required time for drying is 10-25 minutes. Infrared rays are generated through lamps which have filament of 2000 to 2500K.

Factors to be Considered in Infrared Analysis:

1) Distance between Infrared source and dried material
2) Thickness
3) Controlling case hardening of sample
4) Forced Ventilation
5) Weight check Procedures

Figure 2.2: Infrared Moisture Analyzer

Source: Guidance manual by Kett electric laboratory

No standard procedures for this method are issued by AOAC International, but are used based on speed and accuracy of the output.

2.3.4 Rapid Moisture Analyzing Technologies:

These are also known as solid analyzers and are based on thermogravimetric principles. Two types of heaters are available as given below:

1) Halogen heaters
2) Ceramic heaters

Halogen heaters are traded as Mettler Toledo, Columbus etc., These detect moisture levels very quickly. The sample is kept on an aluminium pan and 25 °C to >200 °C is set up. Instrument weighs the moisture and gives the percentage of moisture and total solids. Less time is required for the result. These methods are useful laboratory assays along with production.

2.3.5 Distillation:

This includes co distilling the moisture in food with solvent of higher boiling points. The solvent is not readily miscible with water. Two procedures were used as given below

1) Direct distillation
2) Reflux distillation

Direct distillation involves mineral oil or any other liquid with a flash point which is higher than the boiling point of water. Toluene, benzene and xylene are used mostly in reflux distillations. Advantages include less thermal decomposition, even at higher temperatures. Usage of higher boiling point solvent can increase chemical reactions. Hence solvents of lower boiling points can be used, where time of distillation can be increased.

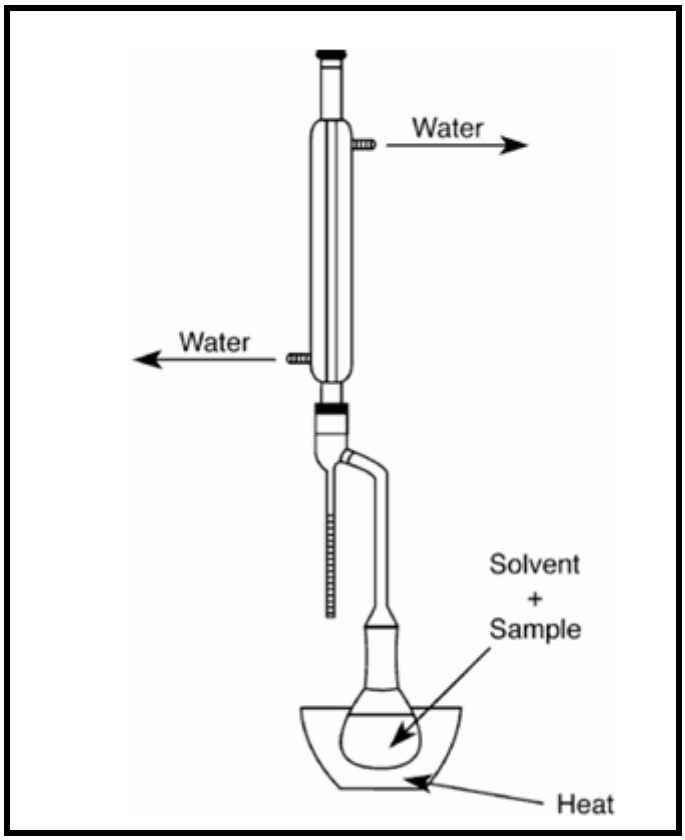


Figure 2.3.1 Apparatus of Reflux Distillation of Moisture

Source: *A Text book on Food Analysis by Suzanne Neilson*

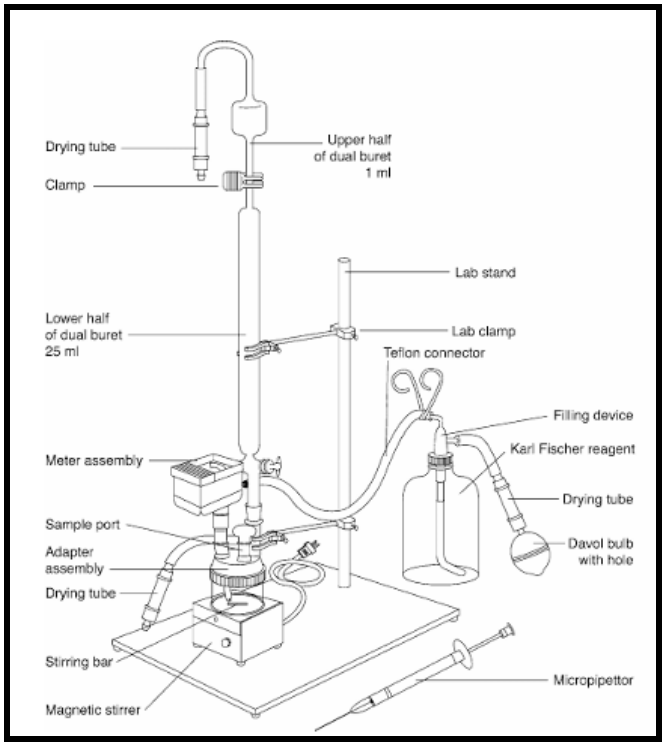
2.3.6 Karl Fischer Titration Method:

This is the chemical method which directly measures moisture content and is used to determine moisture in low moisture foods such as dried fruits and vegetables, chocolates, candies, roasted coffee, oils and fats, high sugar and protein foods. This method uses no heat and has a rapid outcome. The fundamental reaction was based on bunsen theory, which involves reduction of iodine by sulphur in the presence of water.

This titration method was modified where methanol and pyridine were used to dissolve iodine and sulphur. In this, for each one mole of water 1 mole of iodine, 1 mole of sulphur, 3 moles of pyridine and one mole of methanol are used. In a manual titration unit, Iodine and sulphur are added in a closed chamber and titrated. Endpoint should be dark red brown. Instruments can also be used for determining the end point such as potentiometry which increases sensitivity and accuracy.

Disadvantages:

- Incomplete moisture extraction
- External ait in reaction chambers
- Titration unit walls adhered with moisture
- Interference of other food constituents in the reaction
- Over estimation of moisture



2.3.2 Figure-Manual Karl Fischer Titration Unit

Source: *A Text book on Food Analysis by Suzanne Neilson*

2.3.3 Figure-Automatic Karl Fischer Titration Unit (Mettler-Toledo)

Source: *A Text book on Food Analysis by Suzanne Neilson*

2.4 INDIRECT METHODS:

These are mostly Indirect Methods and do not separate moisture from samples. These are non destructive and rapid. These are used in production and quality control. The data should be calibrated and the data obtained from direct methods should be compared.

2.4.1 Dielectric Methods:

Moisture content in foods is directly proportional to electrical properties. It measures the change in capacitance or resistance to an electric current passed through a sample. The instruments used in these procedures require calibration against standard direct method outputs. Sample weight, temperature are the key factors. This is applicable to food systems which contain not more than 30-35% moisture. Principle of this method is that the dielectric constant of water (80.37 at 20 °C) is higher than that of most solvents. Dielectric constant is measured as an index of capacitance.

Figure 2.4.1: Dielectric Constant Meter

Reference: Brookhaven Instruments

2.4.2 Hydrometry:

Measuring specific gravity or density using different principles and measurements is a science and is known as hydrometry. It is highly accurate in terms of output. Different hydrometers along with pycnometers are used. All types of beverages, salt brines and sugar solutions can be tested. Archimedes' principle is the base for measuring specific gravity, which states that a solid suspended in liquid will be buoyed by a force equal to the weight of a liquid displaced. Hydrometer has a standard weight on the end of the spindle, and it displaces a weight of liquid equal to its own weight. For instance, in a liquid with less density, hydrometer sinks to depth and in a liquid of high density, hydrometer will not sink much. The spindle of the hydrometer should be calibrated to read specific gravity specifically at 15.5 °C or 20 °C.

Figure 2.4.2: Hydrometer (Cole-Parmer)

Source: A Text book on Food Analysis by Suzanne Neilson

2.4.3 Pycnometer:

By comparing the weights and volumes of a liquid and water of equal volumes in standardized glassware, specific gravity can be measured. This will give density of the liquid compared to water. Temperatures of both fluids should be the same, when measuring specific gravity. The pycnometer is cleaned and dried at 20°C and should weigh an empty pycnometer. Then it is filled with distilled water at 20°C, then a thermometer is inserted to seal the fill opening to touch last drops and seal the cup to avoid waterflow.

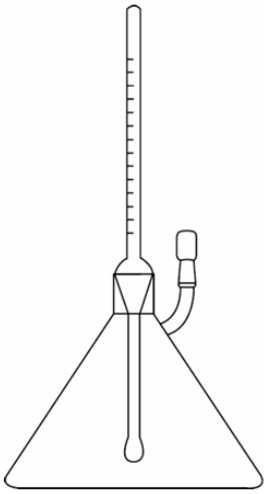


Figure 2.4.3-Schematic Diagram of Pycnometer

Source: A Text book on Food Analysis by Suzanne Neilson

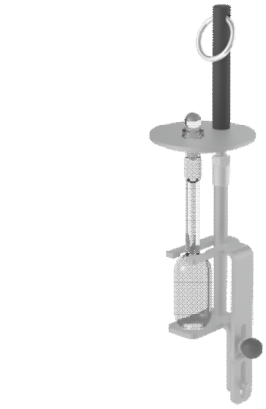


Figure 2.4.4: Bingham Pycnometer with Holder

Reference: Tamson Instruments, 2018

2.4.4 Microwave Absorption:

The microwaves are absorbed at wavelengths of 0.001-1 m and frequency of 0.3-300 GHz. The dielectric constant of permittivity value differences between water and solid dry materials are compared. The approach is based on dielectric constant of permittivity value differences between water and dry materials. The permittivity of dry materials is much lower than water and even minor changes in the amount of water leads to measurable changes in permittivity.

2.5 SUMMARY:

Estimation of moisture in foods is important for processors and consumers for quality in foods. Analytical procedures are very easy but the problem is with obtaining accurate and precise results. Free water can be determined more easily than bound and entrapped water. Some methods involve separation of moisture and some involve chemical and physical

properties. The major problem with many methods is decomposition and removal of water. Precautions should be followed for accurate results. The choice of moisture analysis method, expected moisture and nature of other food constituents are the major factors to be checked thoroughly for precise output.

2.6 TECHNICAL TERMS:

Moisture, Water activity, Hydrometer, Pycnometer, Karl Fischer titration, Permittivity, Conductivity etc.,

2.7 SELF ASSESSMENT QUESTIONS:

1) Why is moisture determination important in foods?
2) Write about methods of Moisture Analysis?
3) Compare Direct and Indirect methods of Moisture Analysis in Foods?

2.8 REFERENCE BOOKS:

1) H.D. Belitz, W.Grosch and P.Schieber. Food Chemistry 4th Revised Edition 2009.
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LESSON-3

DISPERSIONS

3.0 OBJECTIVES:

- To know about importance of dispersions in foods
- To study about dispersed food systems
- To study about types and rheology of dispersions

STRUCTURE:

3.1 INTRODUCTION:

Based on the texture and composition, foods are classified into hard solids, soft solids and liquids. Categories of foods include hard solids, chocolates, biscuits, cheese, soft solids, ice cream, water and honey. Some commercially important foods are also categorized like baby foods, mayonnaise, concentrates, concentrated dispersions, and non-newtonian liquids. In every food processing sector, rheology takes an important part. For example; In Design of handling systems, quality controls, sensory stimuli, viscosity evaluation. Even microstructures of foods also can be determined using rheological properties.

Dispersion is a mechanism in which the continuous phase of other substances, where scattered matter of other particles is dispersed. These include sols, gels, suspensions and also emulsions. While processing these all products, viscoelasticity and shear thinning mechanism plays a major role. When the final product is obtained, the quality is obtained through deformation rates of the product. Dispersion mechanism is well evaluated by the rheometry results. Some foods support the evaluation procedures but food like semi solids exhibit shear thinning mechanisms. These are unstable in nature. However, rheometry is measured with the aid of process equipment, computational fluid dynamics etc.,

TABLE 3.1 CATEGORIES OF FOODS

Categories of Foods	Examples
Hard solids	Biscuits
Soft Solids	Ice-cream
Liquids	Water, Honey

Source: Rheology Reviews, British Society of Rheology, 2004.

3.2 FOODS AS DISPERSION SYSTEMS:

Emulsions, a form of dispersion, are generally unstable and are heterogeneous in nature. Typical thermodynamic properties are seen due to the presence of different phases. In these, an internal phase is dispersed in the other in the form of droplets. The diameter of the particles is higher than 0.1 µm. Not only in foods can be formed emulsions, also with non food materials. But food emulsions must contain only acceptable ingredients for human consumption.

Food emulsions must possess long term stability and generally seen in two forms as given below

1) Oil in Water
2) Water in Oil

Colloidal salts can exhibit unique optical properties, such as the Tyndall effect. When light enters into a colloidal suspension, the dispersed particles scatters light, makes the suspension hazy or milky.

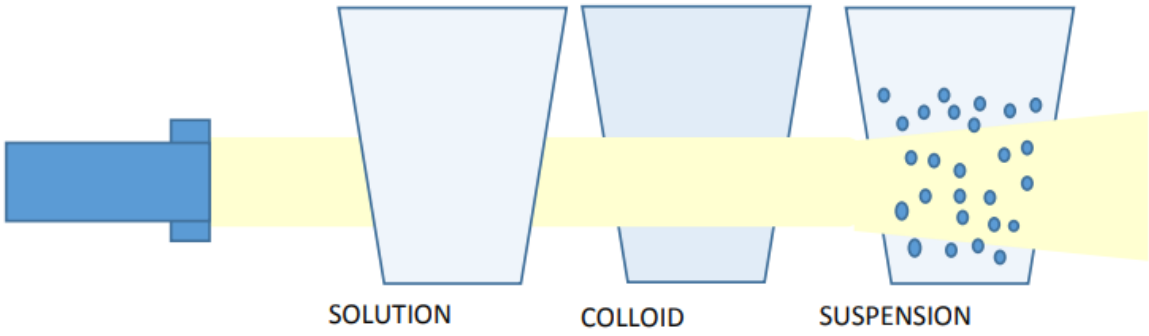

Figure 3.1: Tyndall Effect

Source: Rheology reviews, British society of Rheology, 2004.

In some cases, dispersions are seen as multiple emulsions like water-in-oil-in water and are seen in reduced calorie foods. They don't contain much fat and energy. Stability plays a major role in these types of emulsions. Concentrated emulsions are also seen, which need a compulsory emulsifying agent in processing.

Factors Affecting Stability of Dispersions:

1) Droplet Size
2) Rheology
3) Inter and Intra particle interactions
4) Energy inputs
5) Residence Times
6) Thermal treatments
7) Mixing efficiency
8) Viscosity
9) Viscoelasticity modelling
10) Composition
11) Processing methods
12) Thermal treatments
13) Mixing efficiency
14) High pressure treatments

3.2.1 Composition of Dispersions:

Dispersions are made of different surface active agents to achieve stability. In those egg and milk derivatives are seen mostly. These are traditional surface active agents which also act as emulsifiers. Based on the strength and nature of interactions between emulsifiers, stabilization may differ. Water soluble polysaccharides, hydrocolloids, thickening and structuring agents are also seen in continuous phase. Hydrocolloids are the major components in dispersions. These decrease the mobility of oil droplets and decrease the extension of flocculation process.

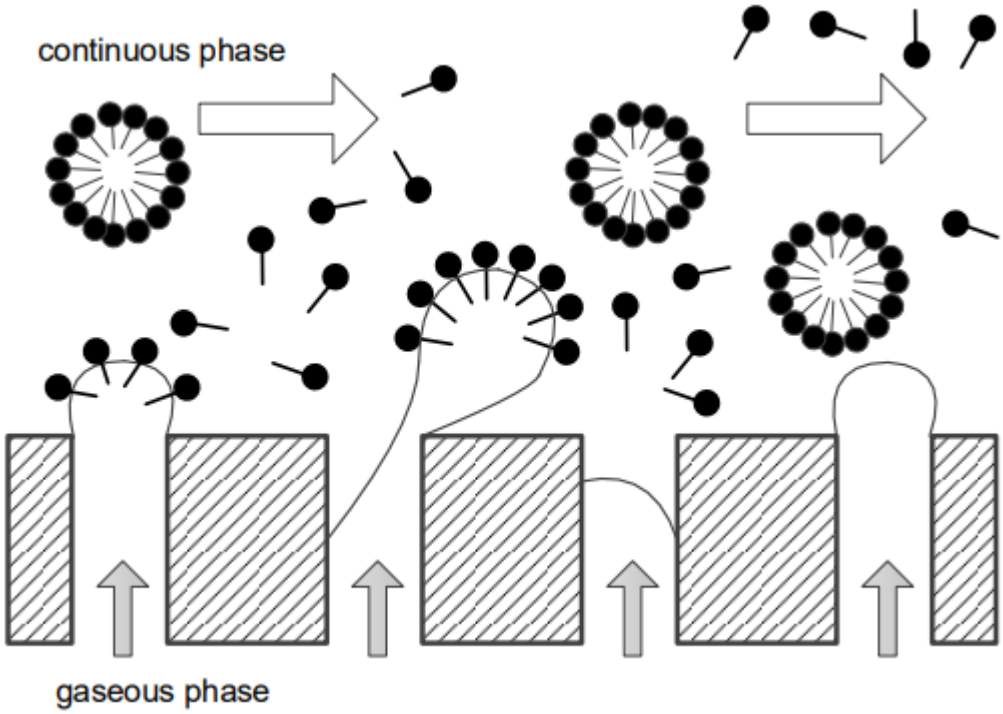

Figure 3.2: Gaseous and continuous phases in dispersions

Reference: Article on A Review on Food Dispersions, Ukraine, 2019

Areas in the Study of Food Dispersions:

1) Processing
2) Microstructure
3) Constitutive equation
4) Computational Fluid dynamics
5) Consumer perception

3.2.2 Processing of Dispersions:

It is a complex unit operation which is influenced by different variables. The processing includes vigorous mechanical energy. Intense agitation has been brought up by consecutive disruption of droplets and coalescence. Droplet size should be measured along with polydispersity. The process is highly affected by viscous and viscoelasticity. Macromolecular and low molecular weight emulsifiers are used for linear relaxation. A rotor and stator turbines are seen with known rotational speed and residence times

A three dimensional network is developed while processing mechanically. Decrease in mean droplet size and polydispersity yields stronger interactions. Creaming was done consequently to yield strong interactions. A non-ionic surfactant, which is a sucrose ester is used which acts as a gel and gives a wide range of concentrations at different temperatures. More increase in agitation speed and emulsification time produces a decrease in droplet size

and polydispersity. Sometimes it may also cause a decrease in values of dynamic viscosity. Balance between larger interfacial surface and gel structure in continuous phase should be achieved during processing. Droplet size also plays a major role on stabilized emulsions.

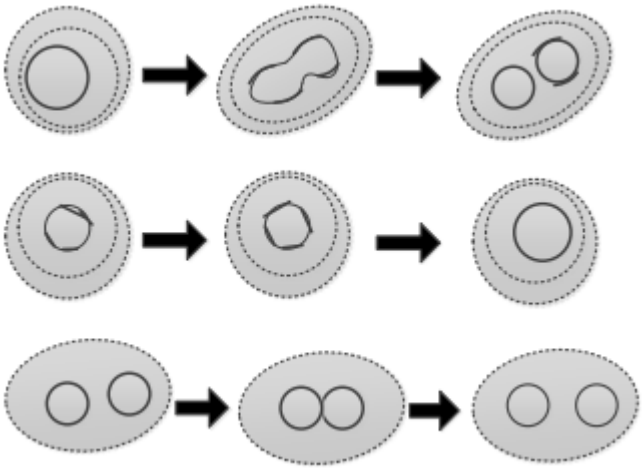

Figure 3.3 Processes During Emulsion Formation

(Reference: Article on A Review on food dispersions, Ukraine, 2019)

Emulsification time should be checked thoroughly when the time is increased, speed also increases. This leads to decrease in particle diameter. This creates an entanglement network and it increases the values of dynamic functions. Emulsion viscosity, firmness and adhesives are also increased in this process. When dealing with protein stabilized emulsions, temperature must be checked. Inter droplet interactions are favoured by increase in temperature and mechanical energy. This might show a difference in protein hydrophobicity. Coalescence process is also increased which gives pea - protein sized emulsions. By giving temperature above 75º C, viscosity increases and after it almost remains constant. At this instance, extensive protein denaturation also may be observed.

Factors Affecting Stability of Emulsion:

1) Interfacial Tension
2) Steric Hindrance
3) Electrostatic Repulsion
4) Stability
5) Surfactant
6) Usage of Lecithin
7) Usage of Mono Diglycerides
8) Usage of Polysorbates
9) Usage of Sodium Stearoyl Lactylate (SSL)

Interfacial Tension:

Usage of emulsifiers will reduce the interfacial tension between oil and water. Coalescence and separation are seen. Lowering interfacial tension gives greater stability

Steric Hindrance:

Emulsifier surround oil droplets as a protecting layer and impedes coalescence. Emulsifier molecules appears physically at the interface

Electrostatic Repulsion:

Generally emulsifiers have electrical charge, causing electrostatic repulsion in oil droplets. Hindrance of droplet aggregation and emulsifier stability enhancement is seen.

Stability:

Based on specific oil and water phases involvement, stability differs. Certain factors like emulsifier concentration, temperature, pH and others influence emulsion stability.

Surfactant:

These play a major role in emulsion stability. Surface tension is reduced and dispersion of liquid phase is also supported. These are hydrophilic and hydrophobic regions which stabilizes emulsion.

Usage of Lecithin:

This is extracted from soybeans or egg yolks. This is used in food and cosmetic products.

Usage of Mono and Diglycerides:

These are esters of glycerol and fatty acids. These are used in baked goods, dairy products.

Usage of Polysorbate:

These are synthetic emulsifiers which stabilizes emulsions and improves texture

Usage of Sodium Stearoyl Lactylate (SSL):

It is a food-grade emulsifier that stabilizes emulsions and improves dough properties. Application is mostly in baked goods.

Sorbitan Esters (Span):

These are known as spans and are used in the food and cosmetics sector.

3.2.3 Rheology of Dispersions:

All types of dispersions, mainly exhibit shear thinning flow behaviours. Especially in plant based concentrated plant food dispersions, these types of behaviours are seen. The minimum shear rate should be applied for better dispersion. The Ostwald-de-Waele model is used to check the viscous flow properties and yield stress. Plant food based and non food suspensions are different from each other in terms of shapes, flexibility and size distributions. Hydration in the continuous food suspension is seen, also known as serum. It is a solution of sugars, organic acids, salts and pectic substances.

Stabilizers made of thixotropic solutions create spatial networks in dispersion medium and form. The network binds the stabilizer and is easily destroyed mechanically and can be easily restored.

Four kinds of systems of two polymers and a solvent are given below

1) Solution of two neutral polymers (Solutions that do not mix)
2) A Neutral polymer and a poly electrolyte (Solutions mixing each other)
3) Like Charged polyelectrolytes (Segregate systems)

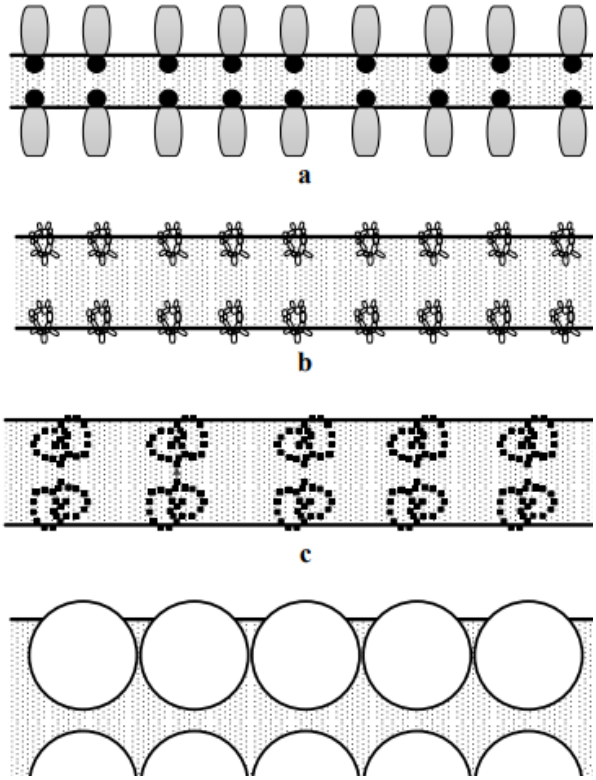

Figure 3.4: Stabilization patterns by surfactants and proteins

Reference: Article on A Review on food dispersions, Ukraine, 2019

The amount and size of insoluble solids depends on the size of the screen obtained. Prior experimentation should be done before determining the dynamic properties. Centrifugation is one of the processes used to study the roles of continuous and dispersed media. Tomato paste, puree and other products' dynamic properties are also determined by using standard centrifugal forces.

Sometimes addition of liquid oils to proteins leads to lower foaming stability. It relates to form destruction and entails rupture of adsorption layers. This also may raise the mechanical strength of foam emulsions. These are considered as best antifoaming agents. These adhere to oil drops and air bubbles depending on size and surface tension. Foam can be destroyed if care is not taken.

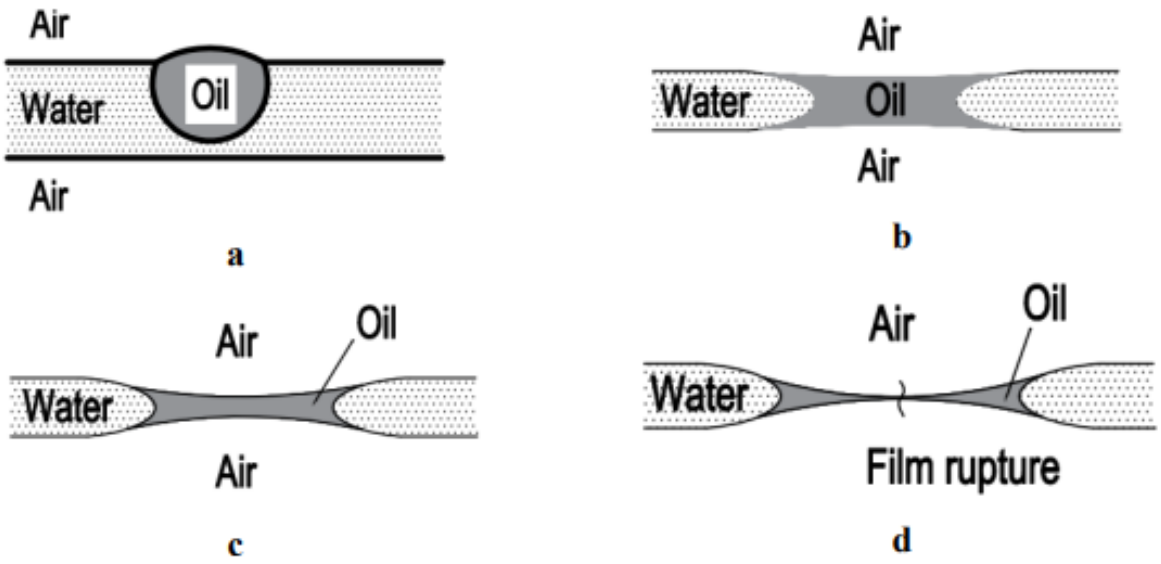

Figure 3.5: Stretching Mechanisms of foam film ruptures

Reference: Article on A Review on food dispersions, Ukraine, 2019

Food dispersions behave differently under applied forces. They shear or deform based on dispersion forms. Flow, deformation characteristics, Viscosity, and elasticity are critical factors that determine the quality of dispersions. The Dispersion term is applicable to emulsions, suspensions and foams. Dispersed particles within a continuous phase exhibit unique behaviours due to specific arrangements and interactions of dispersed particles. Major phenomena associated with food dispersions are given below

1) Viscosity
2) Shear thinning and shear thickening
3) Yield stress
4) Elasticity
5) Thixotropy

Viscosity:

It refers to resistance of fluid to flow. It determines the thickness or fluidity of the product. It generally affects mouthfeel, texture and spreadability. It is influenced by factors like particle size, concentration, temperatures and particulate interactions.

Shear-Thinning and Shear-Thickening:

Non newtonian behaviour is seen with viscosity change in applied share rate. Tomato ketchup exhibits shear thinning behaviour. In some starch based suspensions, viscosity increases with shear rate.

Yield Stress:

It is termed as the minimum amount of stress required to initiate flow of the material. Gels or pastes exhibit yield stress. Examples are yoghurt and mayonnaise

Elasticity:

It refers to the ability of a food product to deform and may return to its own shape, upon force removal. Elastic properties are seen for foams and gels. These properties directly affect the stability, structure, and mouthfeel of the food product.

Thixotropy:

This is a time dependent shear thinning behaviour where decrease in viscosity is seen. Viscosity recovers when shear is removed. Examples are sauces and salad dressings.

Food dispersions are with peculiar behaviour that helps in product formulation, process optimization and quality control. By the help of dispersions, industries achieve desired textural attributes, can measure flow properties and also handle procedures. These are applicable in food, cosmetics and pharmaceuticals etc., Products with more stability and enhanced functional properties are seen. These help to formulate gel based foods. Emulsifiers also play a major role in attaining stable dispersions. Control of processing conditions, incorporation of stabilizers will make the manufacturing process easier and effective.

3.3 SUMMARY:

Dispersions, foams and emulsions have similarities in their production processes but differ in dispersion rate. Rate of surfactant adsorption is also one of the factors that determines quality of dispersions. Emulsions are produced faster than foams. Foam emulsions are difficult to produce and have more demand in food industries. Coalescence is also one indicator for destruction and is determined by the use of surfactants. High usage of proteins, soaked solids, low molecular surfactants or combination of proteins and polysaccharides is recommended to achieve stability of dispersions.

3.4 TECHNICAL TERMS:

Dispersions, Rheology, Dynamic properties, Dispersion media, Dispersed phase, Emulsions, gels, Agitation, Centrifuge, Viscoelasticity etc.,

3.5 SELF ASSESSMENT QUESTIONS:

1) Define dispersions
2) Write about rheology of dispersions
3) Give general categories of foods with examples
4) Write about composition of dispersions

5) Write about processing of dispersions
6) Why is the evaluation of Rheology important in food systems?
7) Define Rheology and give factors affecting rheology.
8) Write about factors affecting stability of dispersion systems.

3.6 REFERENCE BOOKS:

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LESSON-4

COLLOIDS

4.0 OBJECTIVES:

- To know about colloidal systems in foods.
- To study about characteristics of colloids.
- To study about gels, emulsions and foams.

STRUCTURE:

4.1 INTRODUCTION:

Foods are complex systems which contain several components. The quality of food directly depends on components. These components may be in different forms like solids, liquids, solutions, colloid-sols and emulsions. When different conditions are seen, different chemical and physical reactions may occur. Some are desirable and some are undesirable. The principles behind these reactions have to be known to understand and control the changes. Colloidal systems are the special systems, which have major importance in determining food stability. For understanding the colloids, properties, types have to be studied. There are several types of colloidal systems based on the functional and rheological properties of food. Applications of these colloids in different types of foods also play major importance.

Colloidal systems:

Foods contain more moisture, where nutrients are embedded in it. Colloidal states were recognized first by Thomas Graham in the year 1850. Thomas Graham is the father of industry.

According to him, organic compounds are classified into below categories.

1) Colloids
2) Crystalloids

Colloids:

Colloid is derived from the word "Kolla", which is a Greek word. Kolla means glue in the Greek language. It is a system which contains particle size from one mm to 0.1 micron. These may range from 10 to 6 mm and 10 to 4 mm. These are with high molecular weights and they form dispersions with water.

Examples of Colloids:

1) Starch
2) Proteins
3) Glycogen
4) Agar agar

Crystalloids:

The compounds with low molecular weights are known as crystalloids and will create true solutions. Examples of crystalloids

1) Sugars
2) Amino acids

Colloids and solutions are dispersed in water in following states

1) Solids
2) Liquids
3) Gases

Solution:

It is a homogeneous mixture which contains three or more substances. The dissolved substances along with the medium are distributed in the whole solution.

Examples: Salt in water, salt acts as solute and water is the solvent.

Colloidal System:

It is a heterogeneous system. It is also known as dispersion medium or continuous phase. The part in colloidal condition is called dispersion medium or discontinuous phase. In three states of matter, gaseous, solid and liquid sols, Gels and emulsions are seen in colloidal conditions.

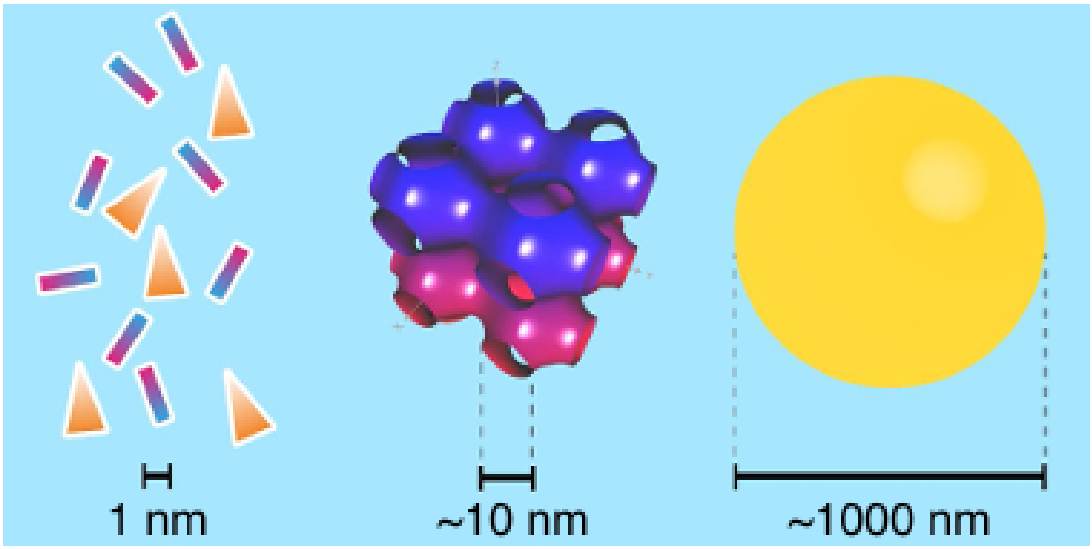

Figure 4.1: Structural Dimensions of Colloids in Foods

Reference: Functional Food Colloids by Linda Hong and Stefan Salentinig, 2022

4.2 CLASSIFICATION OF COLLOIDAL SYSTEMS:

Dispersed systems have peculiar size and are classified based on size of particles. The concept has been elucidated by Thomas Graham. Colloidal systems are classified in to two types

1) One phase dispersions
2) Molecular dispersions
3) Coarse dispersions or suspensions

One Phase Dispersions:

The molecules are dispersed in the system with dimensions below 1 nm.

Molecular Dispersions:

The molecules are dispersed in the system with dimensions ranging from 1 mm to 0.5 mm.

This disperses for very longer times and constitutes a colloidal system.

Coarse Dispersions or Suspensions:

The size is 0.5mm for dispersed particles is seen in this type of systems

These are not restricted to solid or liquid. There is a mixture of gases, liquids and solids. Colloidal systems are classified on the state of the two phases, as given below

1) Dispersed phase
2) Dispersing medium.

Table 4.2: CLASSIFICATION OF COLLOIDAL SYSTEMS

Name	Dispersion Phase	Dispersion Medium	Examples
Fog	Liquid	Gas	Aerosol sprays
Smoke	Solid	Gas	Smoked fish
Foam	Gas	Liquid	Whipped Cream
Emulsion	Liquid	Liquid	Milk
Sol	Solid	Liquid	Skimmed milk
Solid Froth, Foam	Gas	Solid	Bread, idli
Liquid Inclusion	Liquid	Solid	Gelatin, jellies
Solid sol	Solid	Solid	Candles

Reference: Food Chemistry by Fenemma, Fourth Edition.

Gaseous mixture will not create colloidal mixtures. Based on relative affinity of the dispersed phase, the colloidal dispersions are divided into two classes.

1) Lyophilic colloidal systems
2) Lyophobic colloidal systems

Lyophilic Colloidal Systems:

These are water loving colloids. When affinity between phase and medium is high, that dispersion phase is known as lyophilic or hydrophilic, in aqueous dispersion.

Example: Gelatin dispersed in water

Biopolymers

Seaweed gums

Pectic substances

Proteins

Skim Milk

Egg Yolk

Brewed coffee

Table 4.3: COLLOIDAL SYSTEMS

Name	**Examples**
Fog	Aerosols
Smoke	Smoked Tuna
Foam	Whipped Cream
Emulsion	Milk
Sol	Skimmed milk
Solid Froth, Foam	Bread, idli
Liquid Inclusion	Gelatin, jellies
Solid sol	Candles

Reference: Food Chemistry by Fenemma, fourth edition

Lyophobic Colloidal Systems:

In this, the affinity of dispersed phase remains slight and the dispersed phase is known as lyophobic. These are solvent repelling in nature. Water cannot be used as a medium.

Examples: Oils dispersed in water

Butter

Margarine

Lyophobic colloids are aqueous dispersions of inorganic substances and are very rare in food sytems.

4.3 CHARACTERISTICS OF COLLOIDAL SYSTEMS:

Colloidal systems have certain characteristics that differ from solutions. Colloidal systems exhibit certain unique characteristics that help in distinguishing them from solutions. They are given below:

1) Tyndall effect
2) Brownian Movement
3) Electric Charge
4) Adsorption
5) Imbibition
6) Viscosity and plasticity

1. Tyndall Effect:

By using a strong beam of intense light, a solution can be distinguished from dispersion. The beam shows a definite path which is bright. Scattering path of diffusing light rays is seen. The deflection from colloidal particles may be seen. This is called as Tyndall Effect. By the nature of reflections, presence and motion of particles may be identified.

2. Brownian Movement:

By bombardment of millions of molecules in gas or liquid, the colloidal particles show some movement. All the particles are suspended in gas or liquid. This molecular movement is known as brownian movement.

3. Electric Charge:

Colloidal particles are electrically charged. Some colloidal particles carry a positive charge (+), others a negative charge (–). The ionic charge is the same for all the charged particles in a given mass of material. This is why colloidal particles remain in suspension, particles with like charges do not clump together because they are repelled by one another

4. Adsorption:

In this, colloidal particles attract the molecules to the surfaces. In this process, gases, vapours and other matters will come to contact. This is known as adsorption. In this process, particles acquire electric charge and it governs stability of the colloid. Example: Salty soup stock can be modified by addition of egg white

5. Imbibition:

Colloids pick up some water and swell in the presence of water and are known as imbibition. By addition of acids and alkalis, swelling can be improved

6. Viscosity and Plasticity:

Various degrees are seen which offer resistance to pouring. This is known as viscosity. The property of solids enables them to maintain shape under small pressure termed as plasticity.

Environmental factors affecting viscosity and plasticity

1) Temperature:

Viscosity and temperature are inversely proportional to each other. Examples are milk, gelatin and agar.

2) Concentration and aggregation of dispersed particles:

Concentration and aggregation of dispersed particles is directly proportional to viscosity. Examples are cream and protein solids.

4.3.1 Sols:

The system where solid particles are dispersed in liquid is known as sol. Sol differs from the true solution. In True solutions, solutions are separated into molecules and ions that dispersed homogeneously in the solvent. Gelatin is dispersed in water, so sol can be obtained.

When gelatin solution is examined in a microscope, larger individual protein molecules are distinguished from the dispersion medium. Sols flow and are not rigid. When sol is rigid, it is known as gel. No demarcation is seen for sols and gels except in solids and micelles. Sol is fluid and gel is rigid.

In Sol-gel transformation, interlocking of dispersed particles is seen. A three dimensional network is seen. Liquid phase is locked in internal areas of the structure. The entrapment converts sols to gels. When concentration of dispersed phase and temperature are changed, sol can be converted to gel.

When gelatin is dispersed in hot water, immediately it is seen as sol. But the same mixture when cooled forms rigid and transparent gel.

Examples: Fruit jellies

Custards

Gelatin

Weeping of Gel:

Gels loose liquid upon storage and shrinks its structure. This phenomenon is known as syneresis. The liquid that is seen around the product is known as syneresis liquid.

Properties of Sols:

Solid liquid dispersion is known as sol. It contains solid particles which are dispersed in a continuous liquid phase. Example is dispersion of starch in cold water. These exhibit optical properties like opaqueness. These appear clear with the naked eye or even better under a microscope. The dispersed particles are larger to scatter and polarize the light. This is known as the tyndall effect. This is the major property of sols. When viewed under ultra microscope, colloidal particles are seen in irregular motion known as brownian movement. Small particles show more brownian movement than others.

Electrically charged surface is seen on the colloidal particles in sol. This phenomenon is seen only in sol having a continuous aqueous phase. Due to the presence of proteins and phospholipids charge on particles is seen. Selective adsorption of ions is seen when dissolved electrolytes are less. This adsorption stabilizes the colloidal dispersion. However, colloidal particles of the same charge repel each other and reduction in coalesce is seen.

Precipitation of sols is seen due to addition of salts. Charged particles are attracted to polar water molecules and are hydrated in nature. When salt is added, withdrawal of water from particles is initiated and precipitation is seen. Sols have lower osmotic pressure and they can't pass through cellulose.

In water repelling sols, small Interaction between dispersed phase and medium is seen. Viscosity and surface tension are equal as in dispersion medium. salvation of dispersed phase is seen in water loving sols. The viscosity of lyophilic sols is more in dispersion medium and is inversely proportional to temperature and directly proportional to particle concentration. Elasticity is seen at more than 20% concentration.

4.3.2 Gels:

Gel is termed as firmed sol at room temperature. Three major properties of gels are important to check the stability of the food systems. They are strength, brittleness and elasticity. Factors affecting strength are given below:

1) Concentration of gelling agents
2) Concentration of particulates
3) Salt content
4) pH
5) Temperature
6) Presence of polysaccharides
7) Presence of proteins
8) Complex colloids

Caseinate micelles support gels at levels of 10% and gums at 1% concentration. Influence of salts and PH is more adverse and is dependable on concentration. By reducing the number of charged particles, Gel can be transformed to sol. Addition of sugar is also advised for transformation.

Low temperatures reduce mobility and increase viscosity. This also transforms sols to gels. Syneresis or weeping of gel is also seen in some cases. After syneresis, gels may transform to suspensions.

Suspensions:

When solid particles are separated into larger aggregates and dispersed in liquids, they are termed as food suspension. In this, particles sink to the bottom if they are with molecular weight. If weight is less, particles will float. If heated and stirred, suspension can change to gel. Hence we can say that sol suspensions are solid and liquid dispersions.

4.3.3 Foams:

These are also known as colloidal dispersions. These are defined as dispersions of air bubbles in liquid. By agitating liquids, foam is formed. Air is entrapped inside liquid film. Unstable liquid-air interfaces are observed. The air cells are wrapped by liquid films and are considered as continuous phases.

Viscosity and low air-liquid surface tension are the two factors that affect the foaming properties. Films or camellia separates gas bubbles from each other and are elastic. Diameters of foam bubbles range from 1mm to some centimeters. Based on wall thickness, two types of foams are formed.

1) Light Foams
2) Dense Foams

Foams used in cooking are given below and contain more entrapped gas.

1) Whipped cream
2) Ice cream
3) Baked Goods
4) Light breads
5) Meringues
6) Milk foams
7) Gelatin.

The surface area between the gas and liquid phase is very extensive. Opaque appearance of foams is seen due to rigid and elastic walls that reflect light.

Foaming Agents:

These forms foams in a continuous phase prior to dispersion of gas. This gets adsorbed at the surface and provides a distinct surface layer. This stops coalescence of gas bubbles.

Examples of foaming agents are given below

1) Surface active lipids
2) Glycosides
3) Proteins

Methods of formation of foams are of two types

1) Whipping Foams
2) Condensation Foams

Whipping Foams:

It creates bubbles by removing the surface and introduces air to liquids. Repeated whipping gives small bubbles and has fine dispersion. The foams are light in texture

Condensation Foams:

In this, solution is induced with pressure and is released to expand gas bubbles that rise through the liquid. The size of bubbles is big with uneven textured foams.

Factors affecting formation of foams

1) Surface tension
2) Iso-electric point
3) Solubility
4) Low Vapour pressure

Foam Stability:

It is given by two factors. They are drainage and bubble size. More drainage makes the less stable foam. Undesirable foams also can be seen. These are avoided by antifoaming agents. These agents reduce foams and spread a monolayer and displace stabilizing foam films. Undesirable foams are seen in the following products.

1) Fruit juices
2) Coffee extracts
3) Vegetable oils
4) Syrups
5) Fermented products

Silicon oils are used as antifoaming agents. They are water insoluble dimethyl, polysiloxanes.

4.3.4 Emulsions:

These include mayonnaise which are true emulsions. In this, a colloidal dispersion of one liquid in another when both liquids are immiscible is seen. These incorporate characteristics of colloidal systems. The diameter of a particle in dispersed phase is 0.1mm. The liquid in dispersion is known as the continuous phase. Emulsion consists of liquid such as oil, wax, essential oil or water.

Three parts of emulsions are seen. They are dispersed phase, continuous phase and emulsifiers that reduce surface tension of the products. Emulsions are of two types:

1) Oil in water (o/w)- milk, cream, ice cream, mayonnaise, salad dressing
2) Water in oil (w/o)-Butter and margarine

Factors determining quality of emulsions

1) Colour+Opacity
2) Viscosity, Plasticity and Oiliness
3) Flavour

An emulsion can be prepared by mechanical dispersion with the support of beater blades. Emulsifiers should be used to stabilize the emulsion, otherwise stability is not seen. These adsorb at the interface and form water dispersible hydrocolloids. These always have opposite electric charges. Forms of emulsifiers are given below

1) Proteins
2) Gums
3) Gels
4) Fatty Acids
5) Phospholipids

This reduces interfacial tensions between water and oil and are less repellant to each other. Polar end of the emulsifier loves water and the nonpolar end loves oil. Emulsifiers orients in oil in water emulsion. This forms films around tiny drops of oil. Natural emulsifiers like lecithin are also used for emulsification of mayonnaise.

Stabilizers used as Emulsifiers are listed below:

1) Plant Gums
2) Gelatin
3) Lecithin
4) Alginates
5) Seed gums
6) Cellulose derivatives
7) Carboxymethyl celluloses
8) Hydroxypropyl methyl gums
9) Cellulose gums

4.4 SUMMARY:

A colloidal system contains two phases called dispersed phase and dispersing medium. The three states like gaseous, solid and liquid are seen with eight classes of colloidal dispersions. Gases may mix up with solids and liquids. They mostly form solutions rather than colloids. Colloidal particles will always move and contain electrical charges. It has adsorption properties, which is feasible with cooking. Colloidal systems may be lyophilic and lyophobic. Suspensions and sols are liquid and solid dispersions in a continuous phase. In suspension, heavier particles sink and lighter particles rise. Gels are rigid colloid systems. Foams contain entrapped air. Emulsion is a colloidal dispersion with two mutually immiscible liquids. Homogenization is used to stabilize emulsions.

4.5 TECHNICAL TERMS:

Colloids, Lyophobic, Lyophilic, Sols, gels, Foams, Emulsifiers, True Solutions, Solute.

4.6 SELF ASSESSMENT QUESTIONS:

1) Define Colloids?
2) What are sols? Give properties of sols?
3) What are Gels and Foams?
4) Give differences between sols and gels?
5) Write about emulsions and types of emulsions?
6) Write about classification of colloidal systems?
7) Write about applications of colloids?

4.7 REFERENCE BOOKS:

1) Food Chemistry by Fenemma, Fourth Edition.
2) Functional Food Colloids: Studying Structure and Interactions During Digestion by Linda Hong and Stefan Salentinig, 2022, Current Opinion in Food Science 2022, 45:100817.
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Dr. B. Babitha

LESSON-5

STARCH CHEMISTRY

5.0 OBJECTIVES:

After going through this lesson students will understand:

- Types of starches
- Chemical structure of starch
- Properties of different starches

STRUCTURE

5.1 INTRODUCTION:

Starch is a complex carbohydrate predominantly found in cereal grains, tubers, roots, and legumes such as peas, chickpeas, and beans. It serves as a storage polysaccharide, stored within intracellular granules, rather than a structural component in plant cells. During photosynthesis, plants synthesize starch in the chloroplasts of their leaves, storing it as small granules in green tissues. At night, starch is broken down and converted into sucrose, which is then transported to various parts of the plant that require energy or serve as storage organs, such as seeds in cereals and roots in tubers. In these storage tissues, starch is stored as insoluble granules in amyloplasts. Over time, starch accumulates in these granules and is later used as an energy source during germination.

As a vital source of energy and nutrition for both humans and animals, starch plays a significant role in enhancing food quality and nutritional value in the food industry. It is present in a wide range of plants, including green leaves, sago stems, cassava roots, vegetables, fruits (like bananas and plantains), tubers (such as potatoes and cassava), grains like wheat, corn, maize, sorghum, and rice, as well as certain types of algae. Starch is composed of two primary polysaccharides: amylose, which is water-soluble (20-30%), and amylopectin, which is water-insoluble (70-80%).

5.2 TYPES OF STARCHES:

Basically starches are of two types

1) Native starches
2) Processed or Modified starches

5.2.1 Native Starches:

Native or natural starches are derived by extracting starch that occurs naturally in crops such as rice, maize, wheat, potatoes, or cassava. These starches maintain the original structure and characteristics of the plant from which they are sourced. They are commonly used in food processing for texturizing and thickening, in pharmaceuticals, and as raw materials in various industries. Native starches are insoluble in cold water and can swell to varying degrees depending on the temperature applied. They are known for their excellent thickening, gelling, adhesion, and moisture retention properties.

Native starches are long-chain carbohydrates in their pure form. The extraction process produces starch in a white, tasteless, and odorless form, either as a powder or a liquid. These starches are insoluble in cold water and their swelling behavior varies based on the type of starch and the temperature. Unlike modified starches, native starches have not undergone any physical, chemical, or enzymatic treatment. Although they have been used in the food industry for many years, their industrial use is somewhat limited due to certain drawbacks. These include their sensitivity to high temperatures and acidic environments, as starch tends to break down when exposed to heat or acidity, as well as their poor thermal stability. Despite these limitations, native starches possess a wide range of useful properties, including thickening, texturizing, gelling, moisture retention, anti-staling, stabilizing, film-forming, dusting, and dough-binding abilities.

Applications of Native Starch

1) Bakery Mixes
2) Frozen Cakes
3) Sheeted Snacks
4) Batters & Breadings

5) Brewing adjuncts
6) Licorice confections
7) Dry mix soups and sauces
8) Pet foods
9) Processed meat
10) Pudding powders
11) Cold Process Salad dressings and dips
12) Frozen prepared entrée sauces
13) Fruit Preps

5.2.2 Processed or Modified Starches:

Any process that alters the properties of native starch is referred to as starch modification, and the resulting product is called modified starch. This modification is carried out to achieve industrial advantages that cannot be obtained from native starch alone. Starch can be modified through physical, chemical, or enzymatic methods, which lead to changes in its physical and chemical characteristics. Modifications may involve altering the structure or form of the starch granule, or adjusting the shape and composition of the amylose and amylopectin molecules. Modified starches find applications in industries such as food, pharmaceuticals, paper pulp, and textiles. Depending on the specific modification technique, properties such as thickening ability, gelatinization, water retention, palatability, adhesion, and opacity can be adjusted.

The physiochemical and functional properties of native starch are modified through treatments like physical, chemical, enzymatic, or combinations of these methods, in compliance with Good Manufacturing Practices. These modifications aim to enhance the starch's physiochemical characteristics, functionalities, and nutritional properties, or to introduce new properties by altering the molecular structure, such as through cleavage or reorganization. As a result, modified starch exhibits changes in properties like gelatinization temperature, viscosity, retrogradation (when the amylose and amylopectin chains in gelatinized starch realign), gel clarity, texture, and taste, compared to native starch.

Modified starch addresses the limitations of native starch. In the food industry, unmodified native starches are often less desirable due to their low resistance to heat and shear, poor tolerance to acidic and alkaline conditions, lack of resistance to freeze/thaw cycles, instability under shear stress, viscosity loss during processing, and a higher tendency for retrogradation. These weaknesses can lead to a decline in the quality of food products. Therefore, modifying starch is essential to improve its nutritional quality, enhance functionality, and extend shelf life.

Types of Modification Methods: There are four types of modification methods:

1) Physical modification
2) Chemical modification
3) Enzymatic modification
4) Genetic modification

1) Physical Modification:

Physical factors such as moisture, temperature, pressure, pH changes, radiation treatment, and ultrasonic treatment can alter the functional, morphological, physical, and structural properties of starch. These changes can affect attributes like solubility, crystallinity, swelling power, viscosity, and thermal stability. These modifications enable starch to swell and dissolve in cold water.

Several methods are commonly used to modify starch through these physical factors. These include superheating, thermal inhibition treatment, UV and gamma irradiation, microwave treatment, high-pressure treatment, osmotic pressure, instantaneous controlled pressure treatment, mechanical activation using a ball mill, pulsed electric field treatment, micronization in a vacuum ball mill, annealing, and freeze-thaw treatment.

2) Chemical Modification:

Chemical modification of starch involves adding new chemical or functional groups without changing the shape and size of the starch granules, which are deposited in plants in granular form. Starch molecules consist of an hydroglucose unit (AGU) that contains three hydroxyl groups positioned at carbon atoms 2, 3, and 6. This structure makes starch highly reactive, allowing for easy modification through chemical reactions with various functional groups.

Common chemical modification techniques include esterification, etherification, emulsification, cationization, oxidation, acid treatment, and cross-linking, which increases starch’s heat resistance. Traditional chemical methods often use reagents such as acids, acetates, hypochlorites, and phosphates. Cross-linked and stabilized starches are particularly used in food applications. These modifications broaden the range of potential uses for starch in the food industry. However, these chemical processes can be harmful to the environment and often require recycling, which is why enzymatic methods are becoming more widely preferred.

3) Enzymatic Modification:

Enzyme treatment directly modifies the structure of starch, leading to changes in molecular size and weight, the ratio of amylose to amylopectin, and the distribution of branch chain lengths. This process lowers the activation energy and allows the starch's complex chains to break down into simpler sugars, such as maltose, with the aid of enzymes. This is known as enzymolysis, and one of the advantages is that the enzymes can be recovered and reused. This method has largely replaced physical and chemical approaches in the production of modified porous starch. Additionally, enzyme treatment is environmentally friendly and safe for consumers.

One common issue with starchy foods, such as bread and rice cakes, is starch retrogradation, often referred to as staling. To slow down the rate of retrogradation and improve the stability of the gelatinized starch structure, as well as to reduce viscosity loss, various enzymes, emulsifiers, oligosaccharides, and polysaccharides are used.

4) Genetic Modification:

Genetic modification of starch involves the development of amylose-free starch, high amylose starch, or starch with a modified amylopectin structure. In this process, transgenic technologies are used to target specific enzymes within the biosynthesis and degradation pathways of starch in plants.

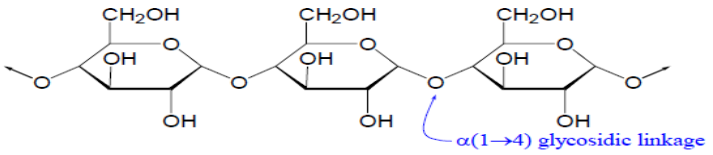
5.3 STRUCTURE OF STARCH:

5.3.1 Chemical Structure: Starch consists of two types of molecules, amylose (20-30%) and amylopectin (70-80%). Both consist of polymers of α-D-glucose units.

Amylose is a linear polymer composed of D-glucose units linked by α-1,4 glycosidic bonds. The side chains of amylose are long and have a high molecular weight. The structure of amylose naturally forms a helical shape. Additionally, amylose can interact with substances like iodine, organic alcohols, and fatty acids, forming complexes known as helical inclusion complexes. When fatty acids are involved, the resulting complex is called an amylose-lipid complex. Amylose can be easily extracted using hot water.

A characteristic property of amylose is its ability to form a gel after the starch granule undergoes cooking, also known as gelatinization and pasting. This behavior is observed in amylose-rich starches such as corn, wheat, rice, and especially high amylose corn starch sourced from hybrid corn varieties. Gel formation occurs due to the reassociation, or retrogradation, of solubilized starch polymers after cooking, which happens more readily with the linear amylose polymer.

Amylose is an unbranched molecule consisting of 200 to 1,000 α-D-glucopyranosyl units connected by α(1-4) glycosidic bonds. It is amorphous by nature and leaches out from starch granules during heating, allowing water to enter and cause swelling. When amylose reacts with iodine, it produces a blue color. It is less soluble in water compared to other starch components and does not form a gel when hot water is added. Hybrid corn, which contains 50% to 70% amylose, is the most common source of amylose. Other sources include rice, potato starches (up to 30%), quinoa, beans, bananas, and lentils.



Amylose

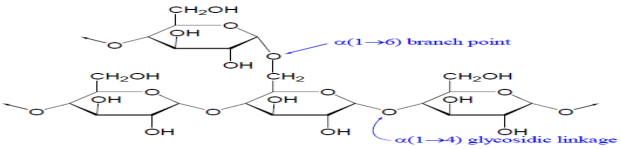
http://www.chtf.stuba.sk/~szolcsanyi/education/files/Organicka%20chemia%20II/Predn aska%209_Sacharidy/Doplnkove%20studijne%20materialy/Carbohydrates_Boudreaux.pdf

Amylopectin:

Amylopectin is a highly branched polymer consisting of 50,000 to 500,000 α-D-glucopyranosyl units connected primarily by α(1-4) glycosidic linkages, with branching occurring at α(1-6) glycosidic bonds. Its molecular weight ranges from 10^7 to 10^8 daltons. Each branch in amylopectin typically contains 20-30 glucose units, and the molecule contains hundreds of these branches. Amylopectin is crystalline in nature, and the branching points contribute to the formation of various allomorphs. The bonds in amylopectin are weaker than those in the linear amylose molecules, which makes it more soluble in water and capable of forming a gel when heated.

Amylopectin also influences viscosity changes upon the addition of water and heat. Each glucose unit in amylopectin contains a maximum of three hydroxyl groups that are available for chemical modification. When amylopectin interacts with iodine, it produces a purple color. It is found in higher concentrations in certain starches, such as waxy corn, waxy potatoes, glutinous rice, and grain rice, while lower levels are present in long-chain rice, amylomaize, and russet potatoes.

As a polymer of α-D-glucose, amylopectin differs from amylose in that it contains a significant number of α-1,6 glycosidic bonds, giving it a branched structure. The molecular structure of amylopectin is highly complex, with chains designated as A, B, and C. The C-chain contains the only reducing group in the molecule, while B-chains serve as side chains that themselves may have additional side chains. The A-chains are the outermost branches that do not carry any branching points.



Amylopectin

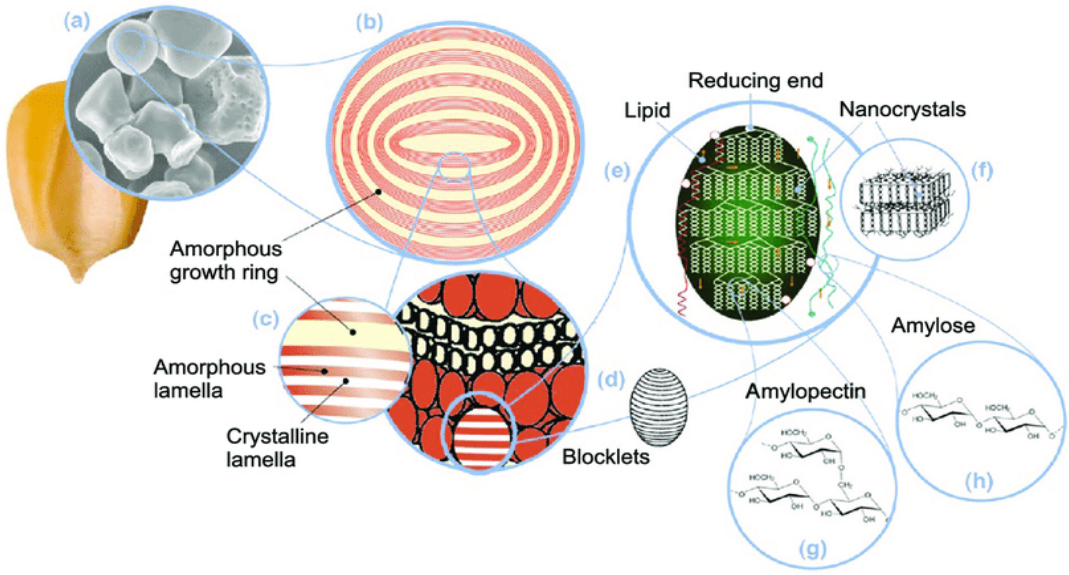
http://www.chtf.stuba.sk/~szolcsanyi/education/files/Organicka%20chemia%20II/Prednaska%209_Sacharidy/Doplnkove%20studijne%20materialy/Carbohydrates_Boudreaux.pdf

Due to the highly branched structure of amylopectin, its properties differ significantly from those of amylose. For instance, the size of the amylopectin molecule and its "tumbleweed-like" shape help slow down retrogradation and can either delay or prevent gel formation. Starches that consist primarily of amylopectin, such as waxy starches, are typically nongelling but tend to have a cohesive and gummy texture.

5.3.2 Physical Structure:

When observed under a light microscope, the starch granule reveals a central area known as the hilum. This region contains a higher water content compared to the rest of the granule. During the processes of drying or wetting, the hilum gradually fades. Starch

biosynthesis begins at the hilum, from which the granules grow. As the granule develops, starch building blocks are added in layers to the outer surface of the growing granule.



a.starch granuels from normal maize b. amorphous and semicrystalline growth rings

c. amorphous and crystalline lamellae d. blocklets constituting a unit of growth rings

e. amylopectin double helices froming the crystalline lamellae of the blocklets

f. nanocrystals

g. amylopectin molecular structure

h. amylose molecular structure.

https://www.researchgate.net/figure/Starch-multiscale-structure-a-Starch-granules-from-normal-maize-30-m-b-amorphous_fig1_314132048

Starch granules exhibit various levels of structural organization at different length scales, including blocklets, lamellae, and lattice spacings, in addition to the growth rings. These growth rings can be either amorphous or crystalline in nature. The amorphous growth rings, which typically have a radial thickness of 120 to 400 nm, are less dense, relatively rich in amylose, and less ordered in terms of amylopectin structure. On the other hand, the semi-crystalline regions consist of alternating amorphous and crystalline lamellae, each approximately 9 to 10 nm thick. The crystalline lamellae are composed of tightly packed amylopectin double helices, while the amorphous lamellae consist of the branching regions of amylopectin.

5.4 PROPERTIES OF DIFFERENT STARCHES:

Starch undergoes various transformations, resulting in different physical structures and properties. Native granular starch can absorb up to 30% of its weight in moisture when

soaked in water, with the absorbed water being retained in the amorphous regions of the starch granules. This absorption process is reversible, as the water can be evaporated through drying at ambient temperatures or below the gelatinization temperature. When starch granules are heated in the presence of water, they eventually lose their double helical crystalline structure in a process called gelatinization. Gelatinization is irreversible, and each type of starch has its own specific gelatinization temperature.

If gelatinized starch is continuously heated with excess water, the granules swell, increase in viscosity, and form a paste. This process is known as pasting. Upon cooling, the viscosity increases as the temperature drops, and starch molecules in the paste form a network, resulting in gel formation. After prolonged storage or repeated freeze-thaw cycles, the starch molecules in the paste or gel crystallize, a process called retrogradation.

The various physical structures of starch make it suitable for a wide range of applications. For instance, granular starch is used in facial powders, while large A-granules of wheat starch are used in carbonless copy paper. Small particle starch or small granular amaranth starch is used in fat substitutes. Starch pastes serve as sizing agents in the paper and textile industries and as thickening agents for soups and canned foods in the food industry. Starch gel is used to make tapioca pudding, desserts, and fillings. Retrograded or crystalline high amylose maize starch is used as resistant starch, which has limited digestibility by humans and is preferred in low-calorie foods. The ratio of amylose to amylopectin in starch plays a crucial role in determining its functionality in food. The amylose and amylopectin content and structure influence the starch granule’s architecture, gelatinization and pasting behavior, as well as its textural properties.

The properties depend on the molecular and structural composition of amylose and amylopectin, percent composition, and arrangement of these two homopolysaccharides in starch granules.

1) It has good iodine-binding ability.
2) It has good swelling power.
3) It lowers water absorption capacity.
4) It has high viscosity.
5) It acts as Emulsifiers.
6) Encapsulants
7) Gelling ability or clouding agent
8) Gelatinization

9) Freeze-thaw and cold storage stabilities

10) Binders

5.4.1 Gelatinization of Starch:

Gelatinization is the process by which starch granules absorb water, swell, and lose their crystalline structure when heated in the presence of water. This irreversible transformation leads to the dissolution of starch granules and the formation of a viscous paste or gel, depending on the concentration and conditions. Gelatinization is a key step in many food and industrial applications, as it affects the texture and viscosity of starch-based products.

Steps in Gelatinization:

1) **Water Absorption**: As starch is heated in water, the granules begin to absorb water, causing them to swell. The absorption of water disrupts the organized crystalline structure of the starch granule.
2) **Granule Swelling**: At a certain temperature, the starch granules reach a critical point where they can no longer maintain their structure. The granules expand and become translucent, which is a visible sign of gelatinization.
3) **Amylose Leaching**: During gelatinization, the linear amylose molecules begin to leach out of the granules and dissolve in the surrounding water, contributing to the thickening of the mixture. Amylopectin, the branched component of starch, remains within the granules, but it also begins to absorb water and soften.
4) **Loss of Crystallinity**: The crystalline regions in the starch granule, composed mainly of amylopectin, start to disorganize, and the starch granule loses its Maltese cross pattern when observed under polarized light.
5) **Formation of Viscous Paste**: As the granules continue to swell and break apart, the mixture becomes increasingly thick and viscous. This thickened liquid is the gelatinized starch paste.

Factors Affecting Gelatinization:

Temperature: Each starch has a characteristic gelatinization temperature, which is the range of temperatures at which the starch granules start to lose their crystalline structure. This temperature varies depending on the source of starch and its composition. For example, potato starch has a relatively low gelatinization temperature, while corn starch requires higher temperatures.

Water Content: Sufficient water is required for gelatinization to occur. Typically, starch requires at least twice its weight in water for effective gelatinization. Without enough water, starch will not fully gelatinize and may become clumpy or form incomplete gels.

pH: The acidity or alkalinity of the starch-water mixture can affect gelatinization. High levels of acidity can weaken the starch structure, whereas alkaline conditions may increase the gelatinization temperature.

Starch Composition: The ratio of amylose to amylopectin within the starch granule influences its gelatinization properties. High amylose starches tend to form firmer gels and may have higher gelatinization temperatures compared to starches with a higher amylopectin content.

5.4.2 Pasting of Starch:

Pasting is a critical property for a wide range of starch applications, such as in thickening agents and sizing agents. The pasting characteristics of starches vary depending on factors like amylose content, lipid composition, and the length of amylopectin's branching chains. For example, starches from cereals, which contain amylose and lipids, tend to have higher pasting temperatures, lower peak viscosities, and less shear thinning than waxy starches. Furthermore, starches with amylose typically exhibit a higher setback viscosity.

The type of starch and its lipid content also play a significant role in determining pasting properties. Normal wheat starch, for instance, has a high level of phospholipids, whereas normal maize starch consists mostly of free fatty acids, some glycerides, and only a small amount of phospholipids. The elevated pasting temperature and relatively low peak viscosity of wheat starch are attributed to the phospholipids, which form a helical complex that interacts with amylopectin molecules and restricts the swelling of the starch granules. In contrast, waxy starches, which contain minimal or no amylose and fewer lipids, typically have lower pasting temperatures and tend to swell more easily, resulting in higher peak viscosities. Ultrahigh pressure treatments can alter the pasting properties of starches, with changes dependent on their lipid content, as well as the molecular and crystalline structures.

Waxy starches, which are composed of 92 to 100% amylopectin, exhibit a high swelling capacity. The greater amylopectin content contributes to their increased viscosity when these starches form pastes. Starches from roots and tubers, such as potato and tapioca starch, also have low lipid content, resulting in lower pasting temperatures and higher peak viscosities. Notably, potato starch has an exceptionally low pasting temperature and a high peak viscosity due to the presence of phosphate monoester derivatives, which carry negative charges that repel each other. This repulsion helps starch granules swell during cooking, promoting a low pasting temperature and high paste viscosity. However, when salts are present, the negative charges on the phosphate groups are neutralized, leading to a considerable reduction in the viscosity of the starch paste.

5.4.3 Retrogradation:

Retrogradation is the process by which starch, after being gelatinized and cooled, undergoes the realignment or reorganization of its molecular structure, primarily the amylose and amylopectin chains. When starch is heated in the presence of water, it swells and forms a gel. Upon cooling, the starch molecules can begin to reassociate, leading to the formation of crystalline structures. This process is referred to as retrogradation and is a natural phenomenon that results in a firmer texture over time.

Retrogradation typically occurs more rapidly with amylose, a linear polymer, compared to amylopectin, which is highly branched. Starches with a higher amylose content tend to undergo more pronounced retrogradation. The rate of retrogradation is influenced by several factors, including the temperature at which starch is gelatinized, the amount of water present, and the specific structure of the starch.

In the food industry, retrogradation can be both beneficial and problematic. For instance, retrograded starch can contribute to the texture of certain food products, such as bread and rice cakes, but it may also lead to staling, a process where the food becomes less desirable in texture. The formation of resistant starch, a type of retrograded starch that is less digestible, can be beneficial in dietary contexts, particularly in low-calorie or high-fiber food products. Resistant starch has potential health benefits, such as improving gut health and aiding in weight management.

Overall, retrogradation is a significant process in starch chemistry that impacts the texture, digestibility, and stability of starch-based foods. It can be managed or modified through various techniques, such as temperature control or the use of additives, depending on the desired outcome in food processing.

5.5 SUMMARY:

Starch is a plant-based polysaccharide primarily found in cereal grains, root vegetables like potatoes, and legumes such as peas, chickpeas, and beans. It is made up of two types of molecules: amylose and amylopectin. The balance between amylose and amylopectin content in a particular starch is crucial for determining its functional properties in food. Starch undergoes various transformations that lead to different physical structures and properties, including processes such as gelatinization, pasting, and crystallization.

5.6 TECHNICAL TERMS:

Amylose, Amylopectin, Retrogradation, Gelatinization, Pasting

5.7 SELF ASSESSMENT QUESTIONS:

1) Write about different types of starches?
2) Explain indetail about starch chemistry?
3) What are the different properties of starch?
4) Discuss the gelatinization of starch?
5) Explain in detail about retrogradation of starch?
6) Describe about pasting property of starch?

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LESSON-6

ANALYSIS OF CARBOHYDRATES

6.0 OBJECTIVES:

After going through this lesson students will understand:

- Method of extraction of starches from different foods
- Determination of reducing and non-reducing sugars
- Estimation of Crude fibre

STRUCTURE:

6.1 INTRODUCTION:

Starch is a homopolysaccharide composed of glucose units and is synthesized by most plant cells. It is primarily stored in seeds (such as cereals and legumes), tubers (like potatoes), roots (e.g., carrots), and certain fruits (e.g., bananas). Starch typically exists in the form of water-insoluble granules. Based on the rate and extent of its digestibility, or the varying velocities and degrees of hydrolysis by α-amylase, starch can be classified into three types:

1) **Rapidly Digestible Starch (RDS)** – Starch that is quickly broken down and absorbed.
2) **Slowly Digestible Starch (SDS)** – Starch that is digested at a slower rate, providing a more gradual release of glucose.
3) **Resistant Starch (RS)** – Starch that resists digestion in the small intestine and functions similarly to dietary fiber.

6.2 METHODS OF EXTRACTION OF STARCHES FROM DIFFERENT FOODS:

6.2.1 Cleaning:

For efficient starch extraction, the raw material must be clean and free from contaminants such as stones, soil, damaged seeds, tubers, roots, yams, and other foreign plant residues. Air aspiration systems are recommended to remove lightweight contaminants, while soil particles adhering to plant material should be removed through scouring and water washing, followed by drying.

6.2.2 Drying:

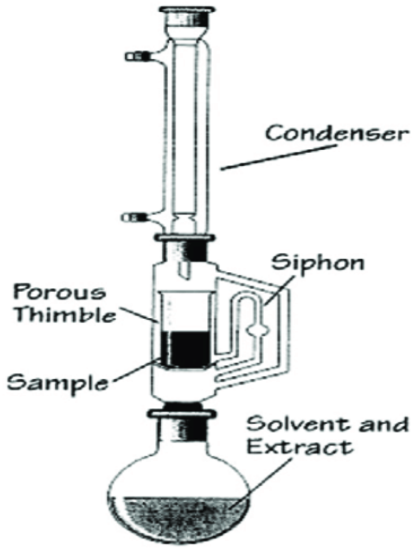
Drying is a crucial step in starch extraction. It should be conducted at lower temperatures to prevent alteration of the physicochemical properties of native starch, as high temperatures may lead to undesirable changes in its structure and functionality.

6.2.3 Crushing:

The initial step in the extraction process involves crushing the raw materials. This increases the surface area, facilitating the release of intracellular polysaccharides. Various crushing methods and instruments have been developed, with one of the most advanced techniques being ultrasonic gas flow crushing technology. This method ruptures the cell wall more effectively, thereby enhancing the efficiency of starch extraction.

6.2.4 Extraction:

Lipids and other materials can be removed from the starch using ethanol reflux in a Soxhlet extractor for 6 to 8 hours. This step ensures the purity of the extracted starch by eliminating non-starch components.



Soxhlet apparatus

https://www.researchgate.net/figure/Soxhlet-extraction-apparatus-set-up-33_fig2_326734330

In the case of pulses, there are two main types of starch extraction: Wet milling and dry milling. Wet milling isolation generally yields high impurity, whereas dry milling is carried out by hammer mills, pin mill, and air classification. Dry milling requires a very high degree of particle size reduction in order to separate starch granules from the protein matrix. The low protein starch fraction gets separated by air classification followed by water washing step to remove the rest of the attached protein. Starch extraction could be assessed by determining starch separation efficiency (SSE) as follows

$$\text{SSE} = \frac{(\%\text{starch in the starch fraction})\,(\%\text{yield of the fraction})}{(\%\text{starch in the flour})}$$

The rate of purity of pulse starches by wet milling is higher than by dry milling. The method is also called isoelectric focusing. The pH used for extraction is between 8.5 and 10.The most commonly used methods for extraction of starch are extraction by dilute alkali-water, and other methods such as DMSO (dimethyl sulfoxide), the organic solvent of alkali metal salt like 2-methoxyethanol-LiCL or acidic aqueous solution.

Hot Water Extraction:

The hot water extraction is the most commonly used procedure which works on the principle that starches have bigger solubility in hot water. Starch is stable in hot water and consequently receives minimal damage by this treatment. The common practice is to extract for 2-6hrs by hot water. If the extract is low in viscosity, then the residue in the extract can be easily filtered. If the extract is viscous then theresidue can be removed using centrifugation.

Extraction by dilute alkali water solution:

This method works on the principle that the solubility of starch is generally higher in dilute alkali solution than in hot water. In this method, NaOH solution or Na_2CO_3 solution of 5 to 15% is used to extract at a temperature below 10^0C. Usually, hot water is first used to extract starch, followed by a dilute solution to extract remaining starch in the residue.

6.2.5 Enzymolysis:

In this method, crushed raw materials are suspended in water, and the optimal conditions for enzymatic reactions, such as temperature and pH, are carefully maintained. A specific amount of composite enzymes is added to the suspension, allowing the reaction to proceed for a designated period. Once the process is complete, the mixture is filtered to separate the residue, and the resulting filtrate contains the extracted polysaccharides. Research indicates that combining **hot water extraction** with enzymatic treatment significantly enhances starch yield by improving the efficiency of polysaccharide release.

6.2.6 Dialysis:

The extracted solution may contain residual impurities, such as inorganic salts, monosaccharides, proteins, fats, and other substances. These impurities can be effectively removed using the **dialysis method**. The dialysis process typically lasts for less than 36 hours, depending on the requirements.

Dialysis bags with specific **molecular weight (MW)** and **molecular weight cut-off (MWCO)** are selected based on the properties of the extract. Before use, these bags require appropriate pretreatment, which involves boiling at around 0.5h to remove impurities, to ensure optimal performance and effective removal of impurities.

6.2.7 Purification Starch:

Several techniques have been developed to purify starch following extraction. These include the **grading precipitation method, salting-out method, metal coordination method, and quaternary ammonium salt precipitation method.**

6.3 DETERMINATION OF REDUCING AND NONREDUCING SUGARS:

6.3.1 Chemical Methods:

Monosaccharides and **oligosaccharides** are reducing agents that can react with various components to form precipitates or colored complexes. These reactions allow for their quantification. The concentrations of both **non-reducing** and **reducing sugars** can be determined by analyzing reducing sugars before and after hydrolysis.

A variety of chemical methods are available for carbohydrate quantification, which can be broadly categorized into three main types:

1) **Titration Methods-**These involve the chemical reaction of sugars with reagents to determine concentration based on measurable end points.
2) **Gravimetric Methods-**These rely on the precipitation of carbohydrates, allowing their mass to be determined after filtration and drying.
3) **Colorimetric Methods-**These methods use the development of color complexes upon reaction with specific reagents, with intensity measured spectrophotometrically to determine sugar concentrations.

6.3.2 Titration Methods:

The **Lane-Eynon method** is a titration-based technique used to determine the concentration of reducing sugars in a sample. In this method, a burette is used to gradually add the carbohydrate solution being analyzed into a flask containing a known quantity of boiling **copper sulfate solution** and a **methylene blue indicator**. As the reducing sugars react with the copper sulfate in the solution, the indicator remains blue. Once all the copper sulfate has reacted, any additional reducing sugars reduce the methylene blue indicator, causing a color change from blue to white. The volume of carbohydrate solution required to reach this endpoint is recorded.

6.3.3 Gravimetric Methods:

The **Munson and Walker method** is a gravimetric technique for determining the concentration of reducing sugars in a sample. In this method, carbohydrates are oxidized under carefully controlled conditions in the presence of heat, an excess of **copper sulfate**, and **alkaline tartrate**. This reaction produces a precipitate of copper oxide (CuO_2).The amount of **copper oxide precipitate** formed is directly proportional to the concentration of reducing sugars in the initial sample.

$$\text{reducing sugar} + Cu^{2+} + \text{base} \rightarrow \text{oxidized sugar} + CuO_2 \text{ (precipitate)}$$

The concentration of the precipitate can be determined using one of two methods:

1) **Gravimetrically**-The precipitate is filtered, dried, and weighed.
2) **Titrimetrically**-The precipitate is redissolved and titrated using a suitable indicator.

6.3.4 Colorimetric Methods:

Anthrone Method:

The **Anthrone method** is a colorimetric technique used to determine the total sugar concentration in a sample. Under acidic conditions, sugars react with the **anthrone reagent**, producing a blue-green color. In this process, the sample is mixed with **sulfuric acid** and the anthrone reagent and then boiled until the reaction is complete. After cooling, the solution's absorbance is measured at a wavelength of **620 nm**. The absorbance is directly proportional to the sugar concentration, providing a linear relationship that facilitates quantification. This method is capable of determining both **reducing** and **non-reducing sugars**, owing to the strong oxidizing properties of sulfuric acid.

Phenol-Sulfuric Acid:

The **Phenol-Sulfuric Acid method** is a widely used colorimetric technique for determining the total carbohydrate concentration in food samples. In this method, a clear aqueous solution of the carbohydrate sample is placed in a test tube, followed by the addition of **phenol** and **sulfuric acid**. Upon reaction, the solution develops a **yellow-orange color**, which results from the interaction between carbohydrates and phenol. The absorbance of the solution is measured at **420 nm**, with the intensity of the color directly proportional to the initial carbohydrate concentration in the sample. The strong oxidizing nature of sulfuric acid converts non-reducing sugars into reducing sugars, allowing this method to quantify the **total sugar content**.

6.4 CRUDE FIBRE:

Dietary Fiber: It refers to plant-derived polysaccharides that are indigestible by humans. Its primary components include **cellulose**, **hemicellulose**, **pectin**, **hydrocolloids**, and **lignin**. Regular consumption of dietary fiber offers numerous health benefits, such as:

- Reducing the risk of **colon cancer**, **cardiovascular disease**, and **constipation**.
- Supporting overall digestive health and promoting well-being.

Certain types of starch, classified as **resistant starch**, are also indigestible by humans and can be analyzed as dietary fiber due to their similar properties and physiological effects.

6.4.1 Major Components of Dietary Fiber:

Cell Wall Polysaccharides: Primary structural component of plant cell wall is celluloses and is universally present in plants. It is typically associated with **hemicelluloses** and **lignins**, and these interactions significantly influence the textural properties of many edible plant materials.

- **Cellulose** is a long, linear **homopolysaccharide** composed of glucose units. The cellulose molecules aggregate to form **microfibrils**, which contribute to the strength and rigidity of plant cell walls.
- **Hemicelluloses** are a diverse group of branched **heteropolysaccharides** containing various sugars in their backbones and side chains. By definition, hemicelluloses are soluble in dilute alkali solutions but insoluble in water.
- **Pectins** are another type of **heteropolysaccharide** found in cell walls. They are rich in **uronic acids**, are soluble in hot water, and possess the ability to form gels.

These polysaccharides play a vital role in determining the structural and functional properties of plant cell walls.

Non-cell wall polysaccharides: Theseare indigestible carbohydrates that do not originate from plant cell walls. These include **hydrocolloids** such as **guar gum**, **locust bean gum**,

gum arabic, **agar**, **alginates**, and **carrageenans**. These polysaccharides are commonly used in food products as **gelling agents**, **stabilizers**, and **thickeners** due to their ability to modify texture and consistency.

Lignin:

Lignin is a complex **non-carbohydrate polymer** composed of approximately 40 aromatic subunits that are covalently linked. Lignin is typically associated with **cellulose** and **hemicelluloses** in plant cell walls, contributing to the structural integrity and rigidity of plants.

6.4.2 Analysis:

There are a number of procedures that are commonly used in many of the methods for dietary fiber analysis:

i. Removal of lipids

Sample Preparation:

- The food sample is first dried to remove any moisture. This ensures that the extraction process focuses only on the lipids and not on water-soluble compounds.
- Once dried, the sample is ground into a fine powder to increase the surface area, making it easier for the solvent to extract the lipids.

Solvent Extraction:

- The ground sample is then treated with a solvent, typically an organic solvent such as hexane or petroleum ether, which is known for its ability to dissolve lipids.
- The sample is either mixed with the solvent and agitated or placed in a Soxhlet extractor, a common apparatus for continuous extraction, where the solvent is vaporized, condensed, and cycled over the sample.
- The solvent dissolves the lipids from the sample.

Separation:

- After extraction, the lipid-solvent mixture is separated from the solid sample, often through filtration or decantation.
- The solvent is then evaporated, leaving behind the extracted lipids, which can be weighed to determine the lipid content of the sample.

ii. Removal of Protein

Proteins are usually broken down and solubilized using enzymes, strong acid or strong alkali solutions. The resulting amino acids are then separated from insoluble fiber by filtration or from total fiber by selective precipitation of the fiber with ethanol solutions.

Protein Breakdown and Solubilization:

- **Enzyme Treatment**: Enzymes like **proteases** (e.g., pepsin, trypsin, or papain) are used to break down proteins into their constituent amino acids and smaller peptides. The choice of enzyme depends on the nature of the protein and the specific analysis requirements.
- **Acid or Alkali Treatment**: If enzymatic breakdown is not used, proteins can be solubilized by treating the sample with **strong acids** (such as hydrochloric acid) or **strong alkalis** (like sodium hydroxide). The acid or alkali denatures the proteins, breaking them down into smaller components, primarily amino acids or peptide fragments.

Separation from Insoluble Fiber:

- After the proteins are broken down and solubilized, the resulting solution contains amino acids and possibly some undigested residues.
- **Filtration**: The liquid is filtered to separate the **insoluble fiber** (which doesn't dissolve in the solution) from the solubilized proteins or amino acids. The fiber, including components like cellulose and lignin, remains on the filter paper while the proteins/amino acids pass through.

Selective Precipitation of Fiber:

- In some cases, **total fiber** (which includes both soluble and insoluble fiber) is selectively precipitated by adding an ethanol solution to the filtrate. Ethanol causes the soluble fiber to precipitate out of the solution.
- The precipitated fiber is then separated by **filtration** or **centrifugation**, leaving the remaining soluble components (such as amino acids) in the supernatant or filtrate.

Analysis:

- The separated **soluble protein or amino acids** can then be analyzed for their quantity and composition.
- **Amino acid analysis** can be done using techniques like **high-performance liquid chromatography (HPLC)** or **ion-exchange chromatography**.
- **Protein content** can be quantified by methods such as **Kjeldahl method** or **Bradford assay**, both of which measure the nitrogen content of the sample.

iii. Removal of starch

Gelatinization of Starch:

1) **Semicrystalline starch** (found in foods like grains and tubers) undergoes **gelatinization** when heated in the presence of water. This process involves the disruption of the crystalline structure of starch granules, causing them to absorb water and swell.

2) **Gelatinized starch** is then more accessible to **enzymatic breakdown** and solubilization.

Breaking Down Starch:

1) The gelatinized starch is typically broken down into **glucose** or **maltodextrins** by specific enzymes, such as **amylase**, which cleaves the starch into smaller sugar units.
2) Alternatively, strong acids (like hydrochloric acid) or alkalis (such as sodium hydroxide) can be used to hydrolyze starch into simpler sugars.

Separation of Glucose and Fiber:

1. Separation of Glucose:

1) Once starch is broken down into glucose or other saccharides, the glucose is separated from **insoluble fiber** (which remains undissolved after the breakdown) by **filtration**.
2) Alternatively, glucose can be separated from **total fiber** (which includes both insoluble and soluble fiber) by adding **ethanol solutions**.

iv. Selective precipitation of fibers

Ethanol-Induced Precipitation**:**Ethanol (in different concentrations) is used to selectively precipitate dietary fibers from the solution. The precipitation occurs because the solubility of monosaccharides, oligosaccharides, and polysaccharides changes with varying ethanol concentrations.

The process works as follows:

1) At **low ethanol concentrations**, **monosaccharides** and **oligosaccharides** (simple sugars) remain soluble in the aqueous phase.

2) As the **ethanol concentration increases**, **polysaccharides** (including dietary fiber) precipitate out of solution due to reduced solubility.

3) Fiber fractions are thus selectively separated from the smaller sugars and other soluble carbohydrates based on their differing solubility in ethanol.

Separation of Fiber:

1) Once the fiber has been precipitated, it can be separated by **filtration** or **centrifugation**.
2) The **soluble sugars** (like glucose and maltodextrins) remain in the filtrate or supernatant, while the precipitated fibers are collected.

Fiber Characterization:

- After precipitation, the **dietary fibers** can be analyzed for their composition and properties, such as **cellulose**, **hemicellulose**, and **lignin** content.
- Techniques like **fiber content analysis** or **spectroscopic methods** may be used for further examination.

v. Water

Monosaccharides, oligosaccharides, some polysaccharides and amino acids are soluble in water. Other polysaccharides and fiber are insoluble in water.

vi. 80% ethanol solutions

Monosaccharides, oligosaccharides and amino acids are soluble; polysaccharides and fibers are insoluble. For this reason, concentrated ethanol solutions are often used to selectively precipitate fibers from other components.

Crude Fiber Method:

The crude fiber method gives an estimate of indigestible fiber in foods. It is determined by sequential extraction of a defatted sample with 1.25% H_2SO_4 and 1.25% NaOH. The insoluble residue is collected by filtration, dried, weighed and ashed to correct for mineral contamination of the fiber residue. Crude fiber measures the cellulose and lignin in the sample, but does not determine hemicelluloses, pectins and hydrocolloids, because they are digested by the alkali and acid and are therefore not collected. It is a simple method to carry out and is the official AOAC method for a number of different foodstuffs.

Total insoluble and soluble fiber method:

The basic principle of this method is to isolate the fraction of interest by selective precipitation and then to determine its mass by weighing. A gelatinized sample of dry, defatted food is enzymatically digested with α-amylase, amylo glucosidase and protease to break down the starch and protein components. The total fiber content of the sample is determined by adding 95% ethanol to the solution to precipitate all the fiber. The solution is then filtered and the fiber is collected, dried and weighed. Alternatively, the watersoluble and waterinsoluble fiber components can be determined by filtering the enzymatically digested sample. This leaves the soluble fiber in the filtrate solution, and the insoluble fiber trapped in the filter. The insoluble component is collected from the filter, dried and weighed. The soluble component is precipitated from solution by adding 95% alcohol to the filtrate, and is then collected by filtration, dried and weighed. The protein and ash content of the various fractions are determined so as to correct for any of these substances which might remain in the fiber:

Fiber = residue weight - weight of (protein + ash).

This method has been officially sanctioned by the AOAC and is widely used in the food industry to determine the fiber content of a variety of foods. Its main disadvantage is that it tends to overestimate the fiber content of foods containing high concentrations of simple sugars, e.g., dried fruits, they get trapped in the precipitates formed when the ethanol is added.

Chemical Methods:

In chemical methods, the fiber content is equal to the sum of all nonstarch monosaccharides and lignins remaining after all the digestible carbohydrates have been removed. The **Englyst-Cummings Procedure** is a useful chemical method for determining the fiber content of food, including soluble and insoluble fiber.

Englyst-Cummings Procedure:

A defatted food sample is heated in water to gelatinize the starch. Enzymes are then added to digest the starch and proteins. Pure ethanol is added to the solution to precipitate the fiber, which is separated from the digest by centrifugation, and is then washed and dried. The fiber is then hydrolyzed using a concentrated sulfuric acid solution to break it down into its constituent monosaccharides. The mass of fiber in the original sample is assumed to be equal to the total mass of monosaccharides present. The concentration of insoluble and soluble dietary fiber can also be determined by this method.

This method can be used to determine the total, soluble and insoluble fiber contents of foods, but does not provide information about the lignin content. This is because lignin is not a polysaccharide, and so it is not broken down to monosaccharides during the acid digestion. For most foods this is not a problem because they have low lignin concentrations. If a food does contain significant amounts of lignin then another method should be used, *e.g.*, the gravimetric method or more sophisticated chemical methods (*e.g.*, the Theander-Marlett method).

6.5 SUMMARY:

Starch can be classified on the basis of the rate and extent of its digestibility or different velocities and degrees of hydrolysis by α-amylase as rapidly digestible starch, slowly digestible starch, and resistant starch. Various methods involved in extraction of starch include hot water extraction, extraction by dilute alkali water solution and enzymolysis. Monosaccharides and oligosaccharides are reducing agents that can react with other components to yield precipitates or coloured complexes which can be quantified. Many different chemical methods are available for quantifying carbohydrates. Most of these can be divided into three categories: titration, gravimetric and colorimetric. The crude fiber is determined by crude fiber method, total insoluble and soluble fiber method, Englyst-Cummings procedure etc.The classification of starch by digestibility provides insights into its metabolic behavior, while various extraction and quantification methods (e.g., titration, gravimetric, and colorimetric) allow accurate analysis of carbohydrates and fibers in foods.

6.6 TECHNICAL TERMS:

Reducing sugars, cell wall polysaccharides, Crude fiber, Solvent extraction, Dialysis

6.7 SELF ASSESSMENT QUESTIONS:

1) What are the various starch extraction methods?
2) How to determine reducing sugars?
3) What are the methods for determination of nonreducing sugars?
4) Explain about various methods used for the determination of crude fiber.

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LESSON-7

LIPID CHEMISTRY

7.0 OBJECTIVES:

After going through this lesson students will understand:

- Nature and types of fats
- Structure of fats
- Physical and chemical properties of different fats and oils
- Chemical changes on rancidity and heating
- Shortening power of fats

STRUCTURE

7.1. INTRODUCTION:

Lipids are organic compounds naturally present in living organisms. They represent a diverse group of substances unified by their characteristic insolubility in water and solubility in organic solvents. Most lipids are derived from fatty acids and serve various biological functions. Certain lipids act as structural components in biological membranes that enclose cells and subcellular structures. Although lipids are present in all foods, their concentration is often below 2%.

Lipids play a vital role in the efficient absorption of fat-soluble vitamins A, D, E, and K in the intestines. Additionally, many enzymes rely on lipids for optimal functionality. In mammals, a significant portion of lipids is stored beneath the skin, where it helps to insulate the body and prevent excessive heat loss to the environment.

Fats provide more than twice the energy per unit weight compared to proteins or carbohydrates, making them a highly efficient energy source. Their presence in food enhances its palatability by contributing to flavor and texture. Lipids stimulate olfactory sensations, delivering taste in the mouth and aroma through the nose, while also creating a desirable texture or "mouthfeel". Furthermore, lipids supply essential fatty acids that humans cannot produce but are critical for growth and overall health.

7.2 NOMENCLATURE:

The nomenclature of lipids refers to the systematic naming of lipid molecules based on their structure and functional groups. Lipids are diverse, and their nomenclature reflects their complexity. Below is an overview of how lipid nomenclature is structured:

1. Fatty Acids:

Fatty acids are carboxylic acids with long hydrocarbon chains. Their names typically include:

- **Systematic Name**: Based on the number of carbon atoms and double bonds, derived from the International Union of Pure and Applied Chemistry (IUPAC) rules. For example:

  Hexadecanoic acid (Palmitic acid, C16:0).

  Octadecenoic acid (Oleic acid, C18:1).

  - **Common Name**: Based on historical or natural sources.
  - **Abbreviation**: Format "C:D", where C is the number of carbons and D is the number of double bonds. Double bonds are further specified by position and configuration (cis/trans).

  Example: **C18:1Δ9 (cis-9-octadecenoic acid)**.

2. Glycerides (Acylglycerols)

These are esters of glycerol and fatty acids.

- **Monoglycerides, Diglycerides, and Triglycerides**: Named based on the number of fatty acid chains.

Example: **Glycerol trioleate** (Triglyceride with three oleic acids).

7.3 CLASSIFICATION:

1) Milk Fats:

Milk fats are the lipids found in the milk of mammals and are an essential component of dairy products.

Characteristics:

- **Composition**:
  - Composed mainly of triglycerides (approximately 98%).
  - Contains short-chain and medium-chain fatty acids like butyric acid and caproic acid, which are rare in other fat sources.
  - Rich in saturated fatty acids, with smaller amounts of monounsaturated and polyunsaturated fatty acids.
- **Physical Properties**:
  - Solid or semi-solid at room temperature but melts at body temperature, contributing to the creamy texture of dairy products.
- **Nutritional Value**:
  - Supplies fat-soluble vitamins (A, D, E, K).
  - Contains conjugated linoleic acid (CLA), which may have health benefits.

2) Animal fats:

Animal fats are lipids derived from the tissues of animals and serve as an important source of energy and structural components.

Characteristics:

- **Composition**:
  - Primarily composed of triglycerides (glycerol + fatty acids).
  - High in **saturated fatty acids** (e.g., palmitic acid, stearic acid) and **monounsaturated fatty acids** (e.g., oleic acid).
  - Contains cholesterol, a sterol found only in animal-derived fats.

Types and Sources:

- **Lard**: Rendered fat from pigs, used in cooking and baking.
- **Tallow**: Rendered fat from cattle or sheep, commonly used in frying, baking, and industrial products like soap.
- **Fish Oils**: High in omega-3 fatty acids (e.g., EPA and DHA), derived from species like cod, salmon, and mackerel.
- **Poultry Fat**: Includes chicken fat and duck fat, known for their flavorful contribution to cooking.
- **Butter**: Although categorized as a milk fat, it is a concentrated form of fat from dairy.

3) Vegetable Fats:

Vegetable fats are lipids extracted from plant sources, commonly referred to as **plant oils** or **vegetable oils**.

Characteristics:

- **Composition**:
  - Predominantly composed of unsaturated fatty acids (both monounsaturated and polyunsaturated fatty acids), such as oleic acid and linoleic acid.
  - Low in saturated fatty acids compared to animal fats.
  - Free of cholesterol but may contain **phytosterols**, which help reduce blood cholesterol levels.
- **Physical Properties**:
  - Most are liquid at room temperature due to their high unsaturated fat content.
  - Some, like coconut oil and palm oil, are high in saturated fats and solidify at cooler temperatures.
- **Nutritional Value**:
  - Rich in essential fatty acids, such as omega-3 (α-linolenic acid) and omega-6 (linoleic acid).
  - Contains fat-soluble vitamins like vitamin E.

7.4 PHYSICAL PROPERTIES:

The physical properties of natural fats and oils are essential for their identification. To ensure accurate identification, multiple properties are typically analyzed, as the characteristics of natural fats and oils can vary. These variations arise due to factors such as climate, soil conditions, and plant variety in the case of vegetable oils, or nutrition, seasonal changes, and other environmental influences for animal-derived fats.

Water Insolubility:

Lipids are hydrophobic, meaning they cannot dissolve in water due to their predominantly nonpolar hydrocarbon chains.

Organic Solvent Solubility:\

Lipids dissolve readily in nonpolar solvents such as ether, chloroform, and benzene.

Density:

Lipids are less dense than water, which causes them to float when in aqueous environments.

Melting Point:

The melting point depends on the type of fatty acids present:

- **Saturated fats** (commonly found in animal sources) have higher melting points.
- **Unsaturated fats** (abundant in vegetable oils) have lower melting points due to their double bonds causing kinks in the structure.

Polarity:

Most lipids are nonpolar molecules, although phospholipids have a polar head and nonpolar tail, giving them amphipathic properties.

Appearance:

Lipids appear as solids (fats) or liquids (oils) at room temperature, depending on their source and fatty acid composition.

Polymorphism:

Polymorphism refers to the ability of a substance to exist in more than one crystalline form. This phenomenon is observed in many long-chain carbon compounds, including fats and oils. The exact number of crystalline forms for each fatty acid or ester is still debated, as some forms have melting points that are so close together, it becomes challenging to separate them. In certain cases, different crystalline forms of the same compound have been identified and are often designated as alpha, beta, and gamma. Understanding polymorphism is crucial when studying the melting behaviors of fats, fatty acids, and their esters, as it helps explain the differences in solidification.

The softening point:

The **softening point** of a fat is sometimes used as an identification method, though it is not applicable to all types of fats. To determine the softening point, capillary tubes are filled with the fat or oil and then placed in ice overnight to allow the substance to solidify and reach equilibrium. The capillary tubes are then secured to a thermometer and submerged in a beaker of water. As the temperature is gradually increased, the temperature at which the fat column begins to rise in the capillary tube is considered the softening point.

The slipping point:

The **slipping point** is a method used to identify certain natural fats and fat compounds. In this procedure, small brass cylinders are filled with solid fat and suspended in a bath near a thermometer. As the bath is gently stirred, the temperature is increased gradually. The **slipping point** is recorded when the fat in the cylinder begins to rise or "slip." This point is influenced by both the amount of air or water incorporated into the fat during its processing and its overall composition.

Specific gravity:

The **specific gravity** of a fat or oil is typically measured at 25°C, although higher temperatures, such as 40°C or 60°C, may be required for fats with higher melting points. During the measurement, the temperature is carefully controlled. The specific gravity of different oils and fats does not vary significantly, but certain factors can influence it. Generally, the **unsaturation** of the fatty acid chains or an increase in the **chain length** of the fatty acid residues tends to raise the specific gravity.

Refractive index:

The **refractive index** refers to the degree of light deflection that occurs when light passes from one transparent medium into another. The refractive indices of fats and oils are commonly measured as they are valuable for identifying these substances and assessing their purity.

An **Abbe refractometer** with temperature control is typically used for these measurements, usually at 25°C. For fats with higher melting points, measurements may be taken at 40°C or even 60°C, although the temperature must be carefully regulated and documented. As the temperature increases, the refractive index tends to decrease. However, the refractive index increases with a longer carbon chain length and a higher number of double bonds in the fatty acid structure.

Smoke and flash points:

The **smoke point** is the temperature at which a fat or oil begins to emit a thin bluish smoke. This is measured using a standard method in an open dish, as specified by the American Society for Testing Materials, allowing the smoke's evolution to be clearly observed and consistently reproduced.

The **flash point** is the lowest temperature at which a liquid fat or oil will release enough vapor to ignite in air. The **fire point**, on the other hand, is the temperature at which the vapor will not only ignite but also sustain combustion.

Fats and oils containing low molecular weight fatty acids generally have lower smoke and flash points. The number of double bonds present in the fatty acids has minimal effect on these temperatures. Smoke and flash points are particularly important for evaluating fats used in frying processes.

7.4.1 Crystallization:

Crystallization refers to the process by which fats and oils transition from a liquid state to a solid or semi-solid state as they cool. During cooling, the fat molecules arrange themselves into an ordered structure, forming crystals. This process plays a critical role in determining the texture and consistency of solidified fats.

The **rate of cooling** and the conditions under which crystallization occurs influence the size and structure of the crystals formed. Faster cooling typically results in the formation of smaller, less organized crystals, whereas slow cooling allows larger, more structured crystals to form. The degree of **unsaturation** in the fatty acid chains also affects crystallization. Fats with higher levels of unsaturated fatty acids tend to crystallize more slowly and may remain softer at lower temperatures, while saturated fats crystallize more easily and form firmer structures.

Crystallization is important in food production, especially in processes like **chocolate making** and the manufacture of **margarine** or **butter**, where desired textures and consistency are achieved through controlled crystallization.

7.4.2 Plasticity:

Plasticity of Solid Fats

Plasticity refers to the ability of a fat or oil to deform under pressure without breaking or flowing. This property is important because it allows fats to maintain a soft yet moldable consistency over a wide range of temperatures. It is particularly significant for fats that need to be spread easily or shaped, such as in the case of butter or margarine.

The plastic nature of fats is a result of the arrangement of **crystals** in a fat mixture. When fats cool, their molecules form crystalline structures that are not rigidly bonded. These crystals can slide past one another, providing the fat with its plastic properties. The **degree of saturation** of the fatty acids in the fat plays a key role in its plasticity. Fats with a high proportion of unsaturated fatty acids (such as vegetable oils) are typically more plastic, while saturated fats tend to be firmer and less plastic. Plasticity is an essential characteristic for fats used in food preparation, as it contributes to the texture, mouthfeel, and spreadability of products.

7.5 CHEMICAL PROPERTIES:

Saponification Number:

It is defined as mg of KOH required to saponify or hydrolyze 1 gm of fat or oil. Saponification number provides the information of average molecular size of the fatty acids present in the given sample. Fats containing more number of short chain fatty acids show higher saponification value.

Iodine Number:

It represents the grams of iodine taken up by 100 grams of fat or oil. Iodine number is a measure of the degree of unsaturation of the fat or oil. The number increases directly with the content of unsaturated fatty acids. Higher iodine number indicates the higher degree of unsaturation of fat and oils.

Reichert-Meisel number:

It is defined as the number of ml of 0.1 N KOH required to completely neutralize the soluble volatile fatty acids contained in 5 gm of fat or oil. It is confined to butter and coconut oil.

The determination of Reichert-Meisel number is important because it helps to detect the adulteration in butter and ghee. Reichert-Meisel value is reduced when animal fat is used as adulterant in butter or ghee.

Polanski number:

It is defined as the number of ml of 0.1 N KOH solution required to neutralize the insoluble fatty acids (not volatile with steam distillation) obtained from 5 gm of fat. Ghee may be adulterated by the addition of insoluble, nonvolatile fatty acids (by addition of animal fat). This can be tested by finding out the Polanski number.

Acid number:

It is defined as a number of mg of KOH required to completely neutralize the free fatty acids present in one gram of fat or oil. Acid number indicates the amount of free fatty acids present in fat or oil. The free fatty acid content increases with age of the fat or oil. The refined oils are free from free fatty acids. But the oils on standing for longer periods decompose due to bacterial lipase or chemical contamination. This gives rise to free fatty acids and decreases the shelf life of fats and increases their acid number which makes them unsuitable for human consumption.

Halogenation:

Halogenation refers to the chemical process where halogen elements-such as **iodine (I)**, **fluorine (F)**, or **chlorine (Cl)**-are added to fats. This process primarily occurs in the presence of **unsaturated fatty acids**, which contain double bonds in their carbon chains. The halogen atoms are added to these double bonds, altering the chemical structure of the fat.

Halogenation is significant in modifying the properties of fats, such as increasing their stability or altering their reactivity. This process is commonly used in various industrial applications, including the modification of oils for specific uses in food or other chemical products.

Acetyl number:

The **acetyl number** is a measure of the amount of free hydroxyl groups (–OH) in a substance, especially in oils, fats, and waxes. It indicates the number of milligrams of potassium hydroxide (KOH) required to neutralize the acetic acid released by the acetylation of the substance. This test is commonly used to assess the degree of unsaturation and the presence of hydroxyl groups in the sample.

7.6 THERMAL DECOMPOSITION:

Thermal decomposition refers to the breakdown of fats and oils when they are exposed to high temperatures. As fats are heated beyond their stability limits, they begin to break down into simpler compounds. This process can result in the formation of volatile gases, fatty acids, aldehydes, ketones, and other by-products.

The temperature at which thermal decomposition occurs depends on the type of fat or oil and its chemical composition. Unsaturated fats, in particular, tend to decompose at lower temperatures than saturated fats. This breakdown can lead to undesirable flavors, odors, and a loss of nutritional quality, especially in cooking oils. Thermal decomposition is an important consideration in cooking and food processing, as it affects the safety, taste, and nutritional value of fats used in frying or baking.

7.7 CHEMISTRY OF FRYING:

The **chemistry of frying** involves complex physical and chemical processes that occur when food is cooked in hot oil. The primary mechanism in frying is heat transfer from the oil to the food, causing moisture inside the food to evaporate. As the moisture escapes, the outer layers of the food become crisp and golden due to the high temperatures of the oil.

During frying, the fat **undergoes several chemical changes:**

1) **Oxidation**: Exposure to heat and oxygen can cause fats to oxidize, leading to the formation of undesirable compounds such as aldehydes and ketones. This process can affect the flavor and nutritional quality of the oil.
2) **Hydrolysis**: Water from the food interacts with the oil, breaking down triglycerides into **free fatty acids** and **glycerol**. The accumulation of free fatty acids can lower the smoke point of the oil and affect the food's taste.
3) **Polymerization**: Over time, especially with repeated use, fats can undergo polymerization, where smaller molecules join together to form larger, more complex structures. This can cause the oil to thicken, become sticky, and lose its ability to fry effectively.

Frying oils must be chosen carefully for their **smoke point**, which is the temperature at which the oil begins to break down and produce smoke. Oils with higher smoke points are preferred for frying to minimize decomposition. Additionally, the **saturation** of the oil (the proportion of unsaturated versus saturated fats) affects the stability and texture of the food being fried. Ultimately, the chemistry of frying not only influences the texture and flavor of the fried food but also impacts the healthfulness and safety of the oil used.

7.8 HYDROGENATION:

Hydrogenation is the process of adding hydrogen to unsaturated fatty acids to convert them into more solid forms, making them more resistant to oxidation. This process is commonly used in food production to transform **polyunsaturated oils**, such as **soybean oil**, into spreadable products like **margarine**. During hydrogenation, hydrogen is forced into the liquid oil, causing some of the unsaturated fatty acids to become saturated. As a result, the oil solidifies and becomes more stable, more spreadable, and less prone to oxidative damage. The hydrogenated oil also exhibits a higher **smoke point**, making it suitable for frying. Additionally, hydrogenated oils are easy to handle, spread, and store.

7.9 INTERESTERIFICATION:

Interesterification is a process that alters the molecular structure of oils and fats by inducing intermolecular ester exchange. Oils and fats are composed of various **triacylglycerol molecules**, each containing different fatty acids in varying positions. When oils or fats are treated with a catalyst like **sodium methoxide** at temperatures around 80°C, the fatty acids are randomly rearranged between the triglyceride molecules, resulting in a change to the oil's physical properties, such as its **melting point** and **consistency**.

One key application of interesterification is the improvement of **natural lard** (pig fat). Natural lard tends to form rough crystals during storage, which can be challenging to handle. This is due to **palmitic acid** being predominantly attached to the **second position** of the triacylglycerol molecules. By randomly redistributing the fatty acids in lard through interesterification, the texture is improved, creating a smoother product known as "**rearranged lard".** Interesterification is also widely used in the production of **margarine**. When **soybean oil** is interesterified with **completely hydrogenated soybean oil**, the resulting product is suitable for making margarine. This rearranged oil is advantageous because it does not contain **trans fatty acids**, unlike oils produced through partial hydrogenation.

7.10 RANCIDITY OF FATS:

Rancidity refers to the spoilage of fats and oils, typically caused by chemical reactions that lead to the development of undesirable tastes and odors. It occurs when the fats or oils are exposed to environmental factors, especially air (oxygen), light, and heat, which initiate a breakdown of the fat molecules.

Types of Rancidity:

Oxidative Rancidity: This is the most common type of rancidity, occurring when unsaturated fats react with oxygen. During this process, **free radicals** are formed, which lead to the oxidation of fatty acids. The breakdown results in the formation of smaller compounds like aldehydes, ketones, and acids, which are volatile and contribute to the characteristic off-flavors and odors. Oxidative rancidity is typically faster in oils with higher levels of polyunsaturated fatty acids.

Hydrolytic Rancidity: Hydrolytic rancidity occurs when water breaks down fats into their component fatty acids and glycerol. This is often caused by the action of enzymes (like **lipases**) or microorganisms that cleave the ester bonds in triglycerides. The free fatty acids released can further undergo oxidation, accelerating rancidity. This type of rancidity is common in oils exposed to moisture or when fats are improperly stored.

Prevention of Rancidity:

1) **Storage in a Cool, Dark Place**: Storing fats and oils in sealed containers away from light and heat can slow down the oxidation process.
2) **Use of Antioxidants**: Adding antioxidants, such as **vitamin E (tocopherol)**, **vitamin C (ascorbic acid)**, and other synthetic compounds like **BHA** (butylated hydroxyanisole) and **BHT** (butylated hydroxytoluene), can help delay oxidation.
3) **Sequestering Agents**: These chemicals bind with trace metals (like iron and copper), preventing them from acting as catalysts for oxidation. **EDTA** and **citric acid** are commonly used.
4) **Hydrogenation**: In some cases, oils can be partially hydrogenated to reduce the number of unsaturated bonds, making them more resistant to oxidation.

7.11 SUMMARY:

Lipids are a heterogeneous group of substances characterized by their insolubility in water and solubility in organic solvents. Most lipids are derivatives of fatty acids. The physical properties of natural fats and oils, such as melting point, slipping point, polymorphism, softening point, and specific gravity, are commonly used for identification. Exposure of fats in food to air causes unsaturated components to undergo chemical changes, resulting in bad odor and taste, a process known as **rancidity**. Frying oils not only transfer heat to cook food but also impart a distinct deep-fried flavor. However, during deep-fat frying, oils undergo chemical deterioration through processes like hydrolysis, oxidation, and polymerization.

7.12 TECHNICAL TERMS:

Polymorphism,Reichert-Meisel number, Saponification number, Iodine number, Halogenation

7.13 SELF ASSESSMENT QUESTIONS:

1) Write about the nature and types of fats?
2) Discuss in detail about physical properties of fats and oils?
3) Write about rancidity?
4) Write about interesterification of fats?
5) Write about importance of shortening power of fats?

7.14 REFERENCE BOOKS:

1) A Text Book of Foods, Nutrition and Dietetics, Third Revised Edition, M.Raheena Begum
2) https://microbenotes.com/lipids-properties-structure-classification-and-functions/
3) http://ecoursesonline.iasri.res.in/mod/page/view.php?id=9128
4) Biochemistry basic and applied by R.A.Fursule, J.S. Kulkarni, P.H.Agarkar
5) Food Chemistry Third Edition Edited by Owen R. Fennema
6) Food chemistry by Satish Parmar, Amit Kumar Jain, K.D.Aparnathi
7) Food Lipids: Chemistry, Nutrition, and Biotechnology Edited by Casimir C.Akoh, David B. Min
8) Nutrition Concepts and Controversies, my Plate Update by Frances Sienkiewicz and Ellie Whitney
9) https://ocw.nagoya-u.jp/files/1/chap3.pdf
10) http://www.iseo.org/httpdocs/FoodFatsOils2016.pdf
11) Understanding Food Principles and Preparation by Amy Brown 4th Edition.
12) https://www.britannica.com/topic/shortening

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LESSON-8

ANALYSIS OF LIPIDS

8.0 OBJECTIVES:

After going through this lesson students will understand:

- Determination of fats in liquid and solid foods
- Method of separation of lipid fraction
- Separation of fatty acids, phospholipids and cholesterol

STRUCTURE:

8.1 INTRODUCTION:

Fats and oils are essential components in both biological systems and food products, playing vital roles in energy storage, structural integrity, and flavor. The analysis of fats and oils is crucial for understanding their composition, quality, functionality, and nutritional value. These substances are primarily composed of lipids, which include triglycerides, phospholipids, and free fatty acids, among others.

The analysis involves identifying the types of lipids present, determining their proportions, and evaluating their physical and chemical properties, such as melting point, iodine value, and saponification value. These characteristics influence the stability, shelf life, and performance of fats and oils in various applications. Additionally, understanding the oxidative stability and susceptibility to rancidity is essential, especially in food and industrial settings.

Modern analytical techniques, such as chromatography, spectrometry, and titration, are employed to assess the quality and purity of fats and oils. These analyses not only ensure compliance with regulatory standards but also aid in product development, process optimization, and the detection of adulteration.

8.2 ANALYSIS OF SOLID AND LIQUID FATS:

Neutral lipids, including triacylglycerols, waxes, and pigments, are readily extracted from tissues using solvents such as ethyl ether, chloroform, or benzene. A commonly used extractant is a mixture of chloroform, methanol, and water in a ratio of 1:2:0.8 (v/v/v). After homogenizing the tissue in this solvent, water is added to the extract, causing it to separate into two phases: a methanol/water top phase and a chloroform bottom phase. Lipids are retained in the chloroform phase, while more polar molecules such as proteins and sugars remain in the methanol/water phase.

The total lipid content of food is typically determined using solvent extraction methods or through alkaline or acid hydrolysis followed by Mojonnier extraction. For multicomponent food products, acid hydrolysis is preferred. Both acid and alkaline hydrolysis methods can be carried out with Mojonnier extraction equipment. The accuracy of direct solvent extraction methods depends on the solubility of lipids in the chosen solvent and the ability to separate lipids from complexes with other macromolecules.

Lipid content determined using one solvent may vary significantly from results obtained with a solvent of different polarity. Beyond solvent extraction, nonsolvent wet extraction methods and various instrumental techniques are also employed to determine fat content. For nutritional labeling purposes, total fat is most commonly analyzed using gas chromatography (GC).

8.2.1 Continuous solvent extraction method:

The **continuous solvent extraction method** is a widely used technique to separate and quantify lipids from food or biological samples. This method ensures efficient lipid recovery by repeatedly exposing the sample to fresh solvent, increasing the extraction yield compared to single-step techniques.

Goldfish Method

Principle: The Goldfish method is a continuous extraction technique in which the solvent continuously flows over the sample to dissolve the fat. The method is highly efficient as it allows constant exposure of the sample to fresh solvent, leading to rapid extraction.

Procedure: The sample is dried, ground, and weighed before being placed into a porous thimble or holder within the apparatus. In the Goldfish apparatus, the solvent flows over the sample continuously, ensuring an uninterrupted extraction process. Suitable organic solvent (e.g., petroleum ether or hexane) is used to dissolve the fat from the sample. The solvent flows through the sample and carries the dissolved lipids to a collection chamber. The solvent is evaporated from the collected mixture, leaving the extracted fat behind for analysis. The evaporated solvent can be condensed and reused. The amount of fat is determined by weighing the residue after solvent evaporation.

8.2.2 Semi Continuous Solvent Extraction Method:

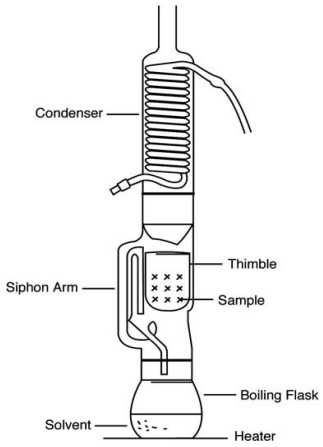
Soxhlet Method: The **Soxhlet method** is a classic laboratory technique used to extract lipids from solid samples. It is a semi-continuous solvent extraction method and is highly effective for isolating fats and oils from various food products, seeds, and other biological samples.

Principle: The Soxhlet method uses a cyclic process in which the solvent is repeatedly vaporized, condensed, and passed through the sample. Lipids dissolve in the solvent, and the process continues until the fat content is completely extracted. The method relies on the solubility of lipids in organic solvents and their separation from non-lipid components.

Procedure: The sample is dried, ground, and weighed to increase surface area and ensure efficient extraction. The Soxhlet apparatus consists of a boiling flask, an extraction chamber (with a porous thimble holding the sample), and a condenser. A suitable organic solvent (e.g., petroleum ether, hexane, or diethyl ether) is placed in the boiling flask.

Extraction Process:

1) The solvent in the boiling flask is heated, causing it to vaporize.
2) The vapor rises through the apparatus and condenses in the condenser, dripping into the extraction chamber.
3) The solvent collects in the extraction chamber and dissolves the lipids from the sample.
4) Once the chamber fills to a certain level, the solvent siphons back into the boiling flask, carrying the dissolved lipids with it.
5) The cycle repeats until complete extraction is achieved.
6) After extraction, the solvent is evaporated, leaving behind the extracted lipids.
7) The extracted lipids are weighed to calculate the fat content.
8) Preparation of samples for further compositional analysis.



Soxhlet Extraction Apparatus

8.2.3 Discontinuous Solvent Extraction Method:

Mojonnier Method:

Principle: Fat is extracted from the sample using a mixture of ethyl ether and petroleum ether in a specialized Mojonnier flask. The extracted fat is dried to a constant weight and expressed as a percentage of the sample's weight. A key advantage of this method is that it does not require prior removal of moisture from the sample, making it suitable for both liquid and solid foods.

When petroleum ether is used to purify the extracted fat, the Mojonnier method closely resembles the **Roese-Gottlieb method**.

Mojonnier flasks are versatile tools used not only for the Mojonnier and Roese-Gottlieb methods but also for performing hydrolysis (acidic, alkaline, or combined) prior to fat extraction. These hydrolyzed samples can then undergo gas chromatography analysis to determine fat content and fatty acid profiles.

The Mojonnier method is primarily applied to dairy products, such as milk, cream, butter, and cheese. However, it is also suitable for analyzing fat content in other foods, including flour and pet food. Both the Mojonnier and Roese-Gottlieb methods involve an initial acid hydrolysis step using hydrochloric acid (HCl), followed by fat extraction with a combination of ethyl ether and petroleum ether.

Chloroform-Methanol Procedure

Principle: The combination of chloroform and methanol is commonly used to extract lipids from various samples. Two prominent methods utilizing this solvent combination are:

1) **Folch Extraction**: Primarily applied to small samples with low moisture content.
2) **Bligh and Dyer Extraction**: A modified version of the Folch method designed for large samples with high moisture content and more efficient solvent usage, especially for low-fat samples.

Additionally, the **Christie modification** replaces water with a 0.88% potassium chloride aqueous solution to improve phase separation and lipid recovery.

Procedure: The food sample is mixed or homogenized in a chloroform-methanol solution at a specified ratio. The homogenized mixture is filtered into a collection tube to separate undissolved solids. A 0.88% potassium chloride aqueous solution is added to the filtered chloroform-methanol mixture. This causes the solution to separate into two distinct layers:

1) **Aqueous Phase (Top)**: Contains water-soluble components.
2) **Chloroform Phase (Bottom)**: Contains lipids.

The two phases are separated using a separatory funnel or by centrifugation. The chloroform layer is collected, and the solvent is evaporated, leaving the extracted lipids. The lipid content is determined by weighing the residue after solvent evaporation.

8.2.4 Non Solvent Wet Extraction Method:

Babcock Method for Milk Fat:

Principle:

The **Babcock method** is a volumetric technique used to determine the fat content in milk. The process involves the digestion of proteins and release of fat through the action of sulfuric acid (H_2SO_4). The fat is separated by centrifugation and quantified by volume in a graduated test bottle. The result is then expressed as a percentage of fat by weight.

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 (Paley bottle) (c) Testing (Courtesy of Kimble Glass Co., Vineland, NJ.)

Gerber method for milk Fat:

Principle:

The principle of the Gerber method is similar to that of the Babcock method, but it uses sulfuric acid and amyl alcohol. The sulfuric acid digests proteins and carbohydrates, releases fat, and maintains the fat in a liquid state by generating heat.

8.2.5 Instrumental methods:

In general, these methods are **rapid**, **nondestructive**, and require **minimal sample preparation** and **chemical consumption**. However, they often come with the drawback of requiring expensive equipment. Additionally, measurements typically necessitate the establishment of **calibration curves**, which must be tailored to specific compositions of the samples being analyzed.

Infrared Method:

Principle:

The **Infrared (IR) method** relies on the absorption of infrared (IR) energy by fat molecules at a specific wavelength of **5.73 µm**. The intensity of the absorption at this wavelength is directly proportional to the fat content in the sample: the higher the energy absorption at 5.73 µm, the higher the fat content.

Applications:

Mid-Infrared (Mid-IR) Spectroscopy:

Milk Fat Analysis: Used in **Infrared milk analyzers** to accurately determine milk fat content in dairy products.

Near-Infrared (NIR) Spectroscopy:

NIR spectroscopy is a valuable tool for rapid, accurate, and non-destructive fat analysis.

Principle:

Near-Infrared (NIR) spectroscopy measures the absorption of light in the near-infrared region (700–2500 nm). It is based on the overtones and combinations of molecular vibrations, primarily associated with C-H, O-H, and N-H bonds, making it particularly useful for analyzing organic compounds, including lipids.

NIR spectroscopy is widely used to measure the fat content of various food products, including meats, cereals, dairy products, and oilseeds. The technique is rapid, non-destructive, and requires minimal sample preparation. Fat content is determined by analyzing the absorbance at specific wavelengths, which correlate to the lipid structure.

NIR spectroscopy is commonly employed in laboratory settings to measure fat content in various food commodities like **Commodities like Meats, Cereals, and Oilseeds**, offering a quick, non-destructive alternative to traditional methods.

Specific Gravity (Foss-Let Method):

Principle:

The **Foss-Let method** determines fat content based on the specific gravity of a sample's solvent extract. The fat content is calculated by measuring how the density of the extracted solution correlates with its fat percentage.

Sample Extraction:

A sample of known weight is extracted for **1.5-2 minutes** in a vibration reaction chamber using **perchloroethylene** as the solvent.

Filtration:

After extraction, the mixture is filtered to remove any solid particles, leaving the fat-containing solvent extract.

Specific Gravity Measurement:

The specific gravity of the extract is measured using a **thermostatically controlled device** with a **digital readout**. This device ensures consistent temperature conditions for accurate results.

Fat Content Calculation:

The specific gravity reading is then used to calculate the fat percentage by referring to a **conversion chart**, which correlates specific gravity values with fat content.

Nuclear Magnetic Resonance

Principle:

Nuclear Magnetic Resonance (NMR) is a nondestructive analytical technique used to measure lipids in food materials. It relies on the interaction of atomic nuclei with a magnetic field, providing detailed information about the molecular structure and composition of substances. NMR is especially effective in determining **lipid melting curves** and measuring **solid fat content**.

Applications:

Solid Fat Content Measurement:

NMR is widely used to determine the solid fat content (SFC) in food products, which is essential for understanding the texture and crystallization behavior of fats in products like margarine, butter, and chocolate.

Total Fat Content:

Low-resolution pulsed NMR can be employed to measure the total fat content in food materials, offering a rapid, reliable alternative to traditional methods.

8.3 SEPARATION OF LIPID FRACTIONS IN FOOD AND FATS:

The lipid content of food commodities, bulk fats, and oils can be characterized by separating and analyzing its various fractions. These fractions include:

- **Fatty acids**
- **Mono-, di-, and triacylglycerols**
- **Phospholipids**
- **Sterols** (e.g., cholesterol and phytosterols)
- **Lipid-soluble pigments**
- **Vitamins**

The analysis of these fractions provides valuable information about the composition, quality, and stability of lipids in food products.

Techniques for Lipid Fraction Analysis

Gas Chromatography (GC):

GC is one of the most commonly used methods to determine various lipid components. It is particularly effective for:

- **Fatty acid composition**: Identifying the types of fatty acids present and their proportions.
- **Fatty acid distribution and position**: Understanding the location of fatty acids within triacylglycerols.
- **Sterol analysis**: Quantifying sterols such as cholesterol and phytosterols.
- **Fat stability and oxidation studies**: Assessing how lipids change over time or under different conditions.
- **Heat and irradiation damage**: Analyzing the impact of processing on lipid quality.
- **Detection of adulterants and antioxidants**: Identifying foreign substances or additives in lipid samples.

GC-Mass Spectrometry (MS):

Combining GC with **mass spectrometry (MS)** provides a powerful tool for identifying and quantifying lipid compounds based on their molecular mass and fragmentation patterns.

High-Performance Liquid Chromatography (HPLC):

Useful for non-volatile components: HPLC is effective for analyzing components that are not easily volatilized, such as:

Hydroperoxides: Important for studying lipid oxidation.

Triacylglycerols: Used to measure the structure and content of triglycerides.

Thin-Layer Chromatography (TLC):

TLC is a widely used method due to its simplicity and affordability. It is often employed for low cost and easy to use. It is used for both qualitative and quantitative analysis.

Qualitative analysis: To determine the presence of specific lipid fractions.

Quantitative analysis: To measure the relative amounts of lipid components by visualizing and comparing the spots.

The separation and analysis of lipid fractions in food and fats are crucial for understanding their composition, stability, and quality. Techniques like **GC**, **HPLC**, **TLC**, and **GC-MS** provide detailed insights into the fatty acid profile, lipid structure, and potential oxidation or adulteration in food products.

Adsorption Chromatography:

Principle:

Adsorption chromatography is a separation technique that relies on the different polarities of compounds in a complex mixture. It uses a stationary phase (adsorbent) and a mobile phase (solvent) to separate the components based on their interactions with the adsorbent material.

In this technique, a polar material, such as **silica gel** (SiO_2), is packed into a column. When a lipid mixture in a non-polar solvent like **chloroform** is applied to the column, the components of the mixture interact differently with the stationary phase based on their polarity.

Procedure:

1) **Column Packing:** A glass column is packed with a polar stationary phase, such as silica gel. The silica gel adsorbs compounds based on their polarity.
2) **Sample Application:** The lipid mixture, dissolved in chloroform, is applied to the top of the column.
3) **Elution of Lipids:**
    - **Neutral lipids**, which are non-polar, pass through the column quickly and are typically eluted first with chloroform.

- **Polar lipids** bind tightly to the silica gel and are eluted using solvents of increasing polarity. The sequence of elution is as follows:
- **Uncharged polar lipids** (e.g., cerebrosides) can be eluted using **acetone**.
- **Highly polar or charged lipids** (e.g., glycerophospholipids) require a more polar solvent, such as **methanol**, to be eluted.

Specific Hydrolysis Aids in Determination of Lipid Structure:

Certain classes of lipids are susceptible to degradation under specific conditions. For example:

1) **Ester-linked Fatty Acids**: Fatty acids in triacylglycerols, phospholipids, and sterol esters can be released by mild acid or alkaline treatment.
2) **Amide-bound Fatty Acids**: These fatty acids, found in sphingolipids, are released under harsher hydrolysis conditions.

Enzymes that specifically hydrolyze certain lipids are valuable tools in determining lipid structure. For instance:

Phospholipases A, C, and D: Each of these enzymes cleaves specific bonds in phospholipids, yielding products with distinct solubilities and chromatographic behaviors.

Phospholipase C: This enzyme releases a water-soluble phosphoryl alcohol (such as phosphocholine from phosphatidylcholine) and a chloroform-soluble diacylglycerol. These products can be characterized separately, providing information on the structure of the intact phospholipid.

By combining **specific hydrolysis** with **product characterization** using techniques like **thin-layer chromatography (TLC)** or **gas-liquid chromatography (GLC)**, the complete structure of a lipid can be determined.

8.4 SUMMARY:

Accurate and precise fat content analysis of food requires understanding the composition of lipids, their physical and chemical properties, and the principles of fat determination. No single standard method is applicable to all food types, and the validity of fat analysis depends on proper sampling and sample preservation before analysis. Prior to analysis, predrying of the sample, particle size reduction, and acid hydrolysis is 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 like 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.

8.5 TECHNICAL TERMS:

Solvent extraction, thin layer chromatography, Gas liquid chromatography, **infrared spectroscopy, GC-Mass Spectrometry**

8.6 SELF ASSESSMENT QUESTIONS:

1) Write in detail about analysis of lipids?
2) Write about various instrumental methods for determination of fats?
3) Explain in detail about the methods for separation of lipid fraction?

8.7 REFERENCE BOOKS:

1) Food Analysis by S. Suzanne Nelsen, Fourth edition.
2) Food Chemistry by Owen R. Fennema, Third edition.
3) Food Analysis by Yeshajahu Pomeranz, Clifton E. Meloan, Third edition.
4) Folch, J., Lees, M., & Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226(1), 497-509.
5) This paper describes the Folch method for lipid extraction, a fundamental reference for organic solvent extraction methods.
6) Christie, W.W. (2003). Lipid Analysis: A Practical Approach (3rd ed.). *Oxford University Press.*
7) This book provides a comprehensive guide to lipid analysis, including methods like GC, HPLC, and TLC.
8) Zhang, S., & Zhang, H. (2019). Advances in lipids analysis by gas chromatography and mass spectrometry. *Food Research International*, 119, 115-128.
9) Focuses on gas chromatography (GC) and mass spectrometry (MS) methods used for lipid analysis, supporting the section on GC-based lipid analysis.
10) Scherer, R., & Wust, M. (2018). Lipid analysis by Nuclear Magnetic Resonance (NMR): A powerful tool for food and nutritional research. *Food Chemistry*, 246, 120-127.

11) Discusses the application of NMR in lipid analysis, particularly its role in quality control and fat content determination.

12) Grob, K., & Mester, Z. (2005). Food analysis by nuclear magnetic resonance. *Analytical and Bioanalytical Chemistry*, 381(4), 982-991.

13) Explores the principles and applications of NMR for lipid analysis, including food quality evaluation.

14) Burdge, G.C., & Calder, P.C. (2005). Lipids in Food: Analysis, Chemistry, and Health Implications. *Journal of Clinical Nutrition*, 81(4), 228-235.

15) Offers insights into lipid composition and analysis methods, suitable for understanding the broader context of fat content determination in food.

16) Sauer, L.C. (1981). Fat in Foods: Determination and Analysis. *Methods in Food Analysis*, 2(1), 139-156.

17) Discusses various methods of fat analysis, including solvent extraction and instrumental methods like GC.

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LESSON-9

NATURE AND TYPES OF PROTEINS IN PLANT FOODS AND EGG

9.0 OBJECTIVES:

After going through this lesson students will understand:

- Nature and classification of proteins
- Types of proteins
- Various proteins present in plants and egg

STURCTURE

9.1 INTRODUCTION:

Proteins are essential macromolecules found in all living organisms, playing a vital role in various biological functions. They are composed of amino acids linked by peptide bonds, forming complex structures that determine their specific functions. Proteins are involved in structural support, enzymatic reactions, immune defence, transportation, and cellular signalling.

Proteins are polymers made from 20 standard amino acids, which determine their structure and function. Their unique three-dimensional shapes arise from interactions such as hydrogen bonding, hydrophobic interactions, and disulfide linkages.

9.2 NATURE OF PROTEIN:

Protein chemistry is the branch of biochemistry that focuses on the structure, function, and interactions of proteins. Proteins are fundamental macromolecules composed of amino acid chains, which fold into specific three-dimensional structures essential for their biological activity. These molecules play critical roles in virtually every biological process, from catalyzing metabolic reactions to providing structural support in cells and tissues.

At the core of protein chemistry is understanding how the sequence of amino acids, also known as the primary structure, determines the protein's final shape and its ability to interact with other molecules. The process by which a protein folds into its functional form, called protein folding, is influenced by a variety of factors including temperature, pH, and ionic strength. Misfolding of proteins can lead to diseases, highlighting the importance of maintaining proper structure.

Proteins can be classified based on their structure and function. Simple proteins are made up of only amino acids, while conjugated proteins contain additional non-protein components such as carbohydrates or lipids. The interaction of proteins with other molecules, like ligands, substrates, and cofactors, is crucial for their functionality and has made protein chemistry a pivotal area of research, especially in drug design, diagnostics, and biotechnology.

As a multidisciplinary field, protein chemistry combines concepts from molecular biology, structural biology, and biophysics. The study of proteins not only deepens our understanding of cellular mechanisms but also facilitates the development of novel therapeutic approaches and industrial applications.

9.3 TYPES OF PROTEINS:

Complete Proteins:

Complete proteins contain all nine essential amino acids in sufficient amounts required by the human body. These proteins are typically derived from animal sources, such as meat, eggs, dairy, and fish. Some plant-based sources, like quinoa and soy, are also considered complete proteins.

Incomplete Proteins:

Incomplete proteins lack one or more of the essential amino acids in sufficient amounts. These are typically found in plant-based sources like legumes, grains, and vegetables. To form a complete protein, incomplete proteins can be combined with other complementary protein sources.

Classification of Proteins Based on Composition:

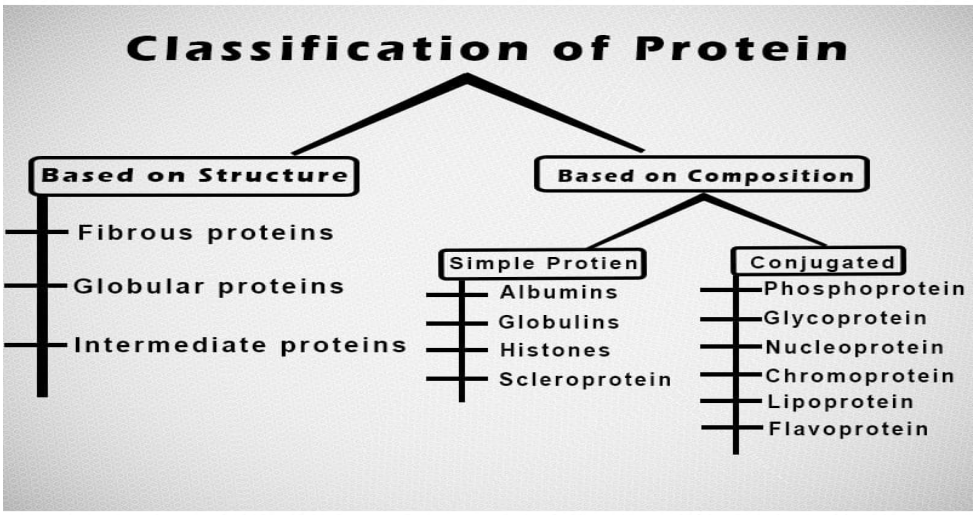

Fig. 9.3.1: Classification of Proteins

Classification of Proteins:

Proteins are complex biomolecules that perform a wide range of functions in living organisms. They are classified based on two major criteria: structure and composition. Below is a thorough explanation of each category, including their properties, examples, and functions.

1. Classification Based on Structure:

Proteins are categorized based on their three-dimensional shape and solubility into three main types: fibrous, globular, and intermediate proteins.

a) Fibrous Proteins:

Fibrous proteins have an elongated, thread-like structure, making them ideal for structural and mechanical support. These proteins are typically insoluble in water and serve protective, supportive, or connective functions in the body.

Characteristics:

- Long, fibrous, and rope-like structures.
- Provide strength, rigidity, and support.
- Mostly composed of repetitive sequences of amino acids.
- Function in structural roles rather than metabolic functions.

Examples & Functions:

1) Collagen-The most abundant protein in the body; found in connective tissues such as skin, tendons, and cartilage.
2) Keratin-Found in hair, nails, and the outer layer of the skin; provides protection and strength.
3) Elastin-Provides elasticity to tissues like blood vessels, lungs, and ligaments.
4) Fibroin-Found in silk; provides tensile strength and flexibility.

b) Globular Proteins:

Globular proteins have a spherical or globular shape and are generally water-soluble, allowing them to perform dynamic roles in biological processes such as catalysis, transport, and immune response.

Characteristics:

- Compact, spherical shape
- Water-soluble, allowing easy transport in the body
- Dynamic roles, including catalysis (enzymes), transport (haemoglobin), and immune defence (antibodies).

Examples & Functions:

1) Haemoglobin-Found in red blood cells, responsible for oxygen transport.
2) Insulin-Regulates blood sugar levels.
3) Enzymes (e.g., Amylase, Protease, Lipase) -peed up biochemical reactions.
4) Antibodies (Immunoglobulins)-Defend against infections by recognizing pathogens.

c) Intermediate Proteins:

Intermediate proteins exhibit characteristics of both fibrous and globular proteins, making them adaptable for various biological functions.

Characteristics:

- Partially structured, flexible proteins.
- Can exhibit both rigidity (like fibrous proteins) and solubility (like globular proteins).
- Play crucial roles in movement, cell structure, and intracellular transport.

Examples & Functions:

1) Myosin-Found in muscle cells, helps in muscle contraction.

2) Fibrinogen-Involved in blood clotting.

3) Tropomyosin-Works with actin in muscle contraction.

Classification Based on Composition:

Proteins can also be classified based on their chemical composition, specifically whether they contain only amino acids or additional non-protein components. This classification includes simple proteins and conjugated proteins.

a) Simple Proteins:

These proteins are made up only of amino acids and do not contain any additional prosthetic (non-protein) groups.

Types of Simple Proteins:

1. Albumins:

- Soluble in water and coagulate upon heating.
- Found in egg white (ovalbumin), blood plasma (serum albumin), and milk (lactalbumin).
- Help maintain osmotic pressure in blood and act as carrier proteins.

2. Globulins:

- Less soluble in water but dissolve in dilute salt solutions.
- Found in blood plasma (immunoglobulins/antibodies), muscle cells (myosin), and soybeans (legumin).
- Play a role in immunity, transport, and muscle function.

3. Histones:

- Positively charged proteins that bind to DNA to form chromatin.
- Found in the cell nucleus.
- Help in DNA packaging and gene regulation.

4. Scleroproteins (Structural Proteins):

- Insoluble in water, providing structural strength.
- Found in collagen (connective tissues), keratin (hair, nails), and elastin (elastic tissues).

b) Conjugated Proteins:

Conjugated proteins contain a non-protein part (prosthetic group) in addition to amino acids. These non-protein components help in specialized biological functions.

Types of Conjugated Proteins:

1. Phosphoproteins:

- Contain phosphate groups.
- Example: Casein (milk protein) – helps in calcium binding.

2. Glycoproteins:

- Contain carbohydrate groups.
- Example: Mucins – found in mucus, providing lubrication and protection.

3. Nucleoproteins:

- Contain nucleic acids (DNA or RNA).
- Example: Ribosomes – essential for protein synthesis.

4. Chromoproteins:

- Contain coloured prosthetic groups (pigments).
- Example: Haemoglobin (red due to heme) and chlorophyll (green pigment in plants).

5. Lipoproteins:

- Contain lipid (fat) molecules.
- Example: HDL (High-Density Lipoprotein) & LDL (Low-Density Lipoprotein) – involved in cholesterol transport.

6. Flavoproteins:

- Contain flavin molecules (such as FAD, FMN).
- Example: Succinate dehydrogenase – involved in cellular respiration.

9.4 PROTEINS IN PLANT-BASED FOODS:

Plant-based foods, unlike animal foods, are typically classified as containing incomplete proteins. This means they often lack one or more of the essential amino acids. However, a varied plant-based diet can provide all the amino acids needed for good health.

Sources of Plant-Based Protein

Here's a more detailed look at different plant-based protein sources:

1. Legumes: (e.g., beans, lentils, chickpeas, peas)

- Legumes are among the richest plant-based protein sources. They are also high in fibre, making them good for digestion and heart health.
- Protein content: Around 15-25 grams of protein per cooked cup.

2. Grains: (e.g., quinoa, rice, oats, barley, buckwheat)

- While grains are generally lower in protein than legumes, some grains like quinoa and amaranth are considered complete proteins since they contain all nine essential amino acids.
- Protein content: Around 4-8 grams of protein per cooked cup (for quinoa and other whole grains).

3. Nuts and Seeds: (e.g., almonds, walnuts, chia seeds, flaxseeds, pumpkin seeds)

- Nuts and seeds are not only protein-rich but also provide healthy fats, vitamins, and minerals. For example, chia seeds and hemp seeds are excellent sources of plant-based protein.
- Protein content: Around 5-8 grams of protein per ounce.

4. Soy Products: (e.g., tofu, tempeh, edamame)

- Soy products are the best-known plant-based sources of complete proteins. Tofu and tempeh are very versatile in cooking and are popular protein replacements in plant-based diets.
- Protein content: Tofu can provide 10 grams of protein per 4 oz, while tempeh offers around 15 grams per 4 oz.

5. Leafy Greens and Vegetables: (e.g., spinach, broccoli, Brussels sprouts)

- While vegetables don't usually contain as much protein as legumes or grains, many leafy greens like spinach contain a surprising amount of protein per calorie. These also provide fibre, vitamins, and minerals.
- Protein content: Spinach contains around 5 grams of protein per cooked cup.

6. Seitan (Wheat Gluten):

- Seitan is a high-protein meat substitute made from gluten, the protein in wheat. It has a chewy, meat-like texture and is often used in vegan and vegetarian cooking.
- Protein content: Around 21 grams of protein per 3.5 oz serving.

7. Peas and Pea Protein:

- Peas, like split peas and garden peas, are great sources of protein, and pea protein is often used in plant-based protein powders and meat alternatives.
- Protein content: About 9 grams per cooked cup of peas.

8. Other Plant-Based Protein Sources:

- **Nutritional yeast** (high in B vitamins and protein)
- Chia seeds, hemp seeds, and pumpkin seeds
- Spirulina, a blue-green algae, is another high-protein option that is sometimes used in smoothies and snacks.

9.5 EGG PROTEINS:

In protein chemistry, egg proteins are highly valued for their nutritional quality and functional properties. Egg proteins, particularly those found in egg whites (albumen) and egg yolks, play essential roles in various biological functions and have distinct biochemical characteristics. Here are the main types of proteins found in eggs:

1. Egg white proteins (albumen proteins)

Egg whites, or albumen, are primarily composed of water (about 90%) and proteins (about 10%). The proteins in egg whites have diverse functions, including structural support and defense against bacterial infection. Key egg white proteins include:

A. Ovalbumin:

- Function: Ovalbumin is the most abundant protein in egg white, constituting about 54% of the total protein content. It serves as a storage protein and provides amino acids for the developing embryo.
- Properties: It is water-soluble and forms a gel-like structure when denatured, which is essential in various food applications, especially in the formation of foams during cooking or baking.

B. Ovo transferrin (Conalbumin):

- Function: Ovo transferrin accounts for about 12-13% of the total protein in egg white and has antimicrobial properties due to its ability to bind iron, thereby preventing bacterial growth.
- Properties: It is an iron-binding glycoprotein and has antioxidant properties. It also plays a role in transporting metal ions within the egg.

C. Ovomucoid:

- Function: Ovomucoid is a major egg white protein that acts as a protease inhibitor, preventing the degradation of other proteins within the egg.
- Properties: It is a glycoprotein and has anti-inflammatory effects. It also contributes to the gel-like texture of egg white when denatured.

D. Lysozyme:

- Function: lysozyme is an enzyme with antibacterial activity, helping to protect the egg from pathogens.
- Properties: It hydrolyzes bacterial cell walls and is part of the egg's innate immune system. Lysozyme is also used in food preservation due to its antimicrobial properties.

E. Avidin:

- Function: Avidin is a biotin-binding protein that binds tightly to biotin (vitamin b7), preventing bacterial growth in the egg.
- Properties: Avidin can inhibit the absorption of biotin in the digestive system when consumed raw but becomes harmless when cooked. It is also used in biochemical research applications to isolate biotin-labelled compounds.

F. Glycoproteins:

- Function: These proteins have carbohydrate groups attached and are involved in structural integrity and defense mechanisms within the eggs.
- Properties: They include proteins like ovoglobulins and have roles in stabilizing egg white proteins and influencing their gel-forming properties.

G. Ovoglobulins:

- Function: These are globular proteins found in the egg white that help form the foam structure when the egg is beaten or whipped.
- Properties: Ovoglobulins contribute to the egg’s ability to act as a foaming agent in culinary applications like meringues, soufflés, and whipped egg whites.

2. Egg yolk proteins:

Egg yolk proteins, although less abundant in comparison to egg white proteins, play important roles in the nutritional composition of eggs. The proteins in egg yolk are involved in lipoprotein functions, nutrient transport, and immune responses. Key egg yolk proteins include:

A. Vitellogenin:

- Function: Vitellogenin is a precursor protein synthesized in the liver and transported to the yolk, where it is cleaved into smaller proteins. It serves as a major source of nutrients for the developing embryo.
- Properties: It is a phospholipoprotein that binds lipids and aids in the transport of nutrients to the yolk.

B. Phosvitin:

- Function: Phosvitin is a phosphoprotein that binds metal ions, particularly iron and zinc, and contributes to the yolk's function as a nutrient reservoir.
- Properties: It has a high phosphate content and is important in the regulation of metal ion homeostasis in the yolk.

C. Livetin:

- Function: Livetin is a family of proteins found in egg yolk that have enzymatic and transport functions.
- Properties: There are three types of livetin—α-livetin, β-livetin, and γ-livetin—which are involved in metabolic processes and immune responses in the egg.

D. Lipoproteins:

- Function: Lipoproteins in the egg yolk, such as low-density lipoprotein (LDL) and high-density lipoprotein (HDL), are responsible for the transport of lipids and fat-soluble vitamins.
- Properties: They are essential for providing the developing embryo with energy and fat-soluble nutrients.

3. Other Functional Proteins:

In addition to the main proteins mentioned above, egg proteins are utilized for various functions in food products and industries due to their unique functional properties:

A. Egg White Proteins in Food Applications:

- Foam formation: proteins like ovalbumin, ovomucoid, and ovoglobulins contribute to the formation of stable foams, which are used in baking, meringues, and marshmallows.
- Emulsification: some egg proteins, especially those found in egg yolk, function as emulsifiers, which allow oil and water to mix. This property is crucial in making products like mayonnaise and salad dressings.

B. Egg Proteins in Biotechnology:

- Avidin: due to its high affinity for biotin, avidin is used in molecular biology and immunology for applications such as biotin-streptavidin affinity systems.
- Lysozyme: it is also used in the food industry as a preservative due to its antimicrobial properties.

9.6 SUMMARY:

Plant foods and egg proteins are both valuable sources of nutrition, offering unique benefits for a balanced diet. Plant-based foods, such as legumes, grains, nuts, seeds, fruits, and vegetables, provide essential nutrients, including fiber, vitamins, minerals, and antioxidants, which support digestion, heart health, and overall well-being. However, most plant proteins are considered incomplete, meaning they lack one or more essential amino acids. To ensure a complete protein intake, combining different plant sources, such as beans with rice or lentils with whole grains, is recommended.

On the other hand, egg proteins are considered one of the highest-quality animal proteins due to their complete amino acid profile, high bioavailability, and excellent digestibility. Eggs contain all nine essential amino acids in optimal proportions, making them an ideal source of protein for muscle growth, tissue repair, and overall body function. Additionally, eggs provide essential nutrients like choline, which supports brain health, and lutein and zeaxanthin, which benefit eye health. While plant-based proteins contribute to a healthy and sustainable diet, incorporating eggs can enhance protein quality, particularly for those needing complete amino acid coverage. A balanced approach, including both plant-based proteins and egg proteins, ensures comprehensive nutrition and promotes overall health.

9.7 TECHNICAL TERMS:

Derived lipids, Avidin, and Ovalbumin.

9.8 SELF-ASSESSMENT QUESTIONS:

1) What are the different types of proteins?
2) Explain about proteins present in plant foods?
3) Discuss about proteins present in eggs and their importance in health?

9.9 REFERENCE BOOKS:

1) Applied Food Protein Chemistry Edited by Zeynep Ustunol.

2) Food Chemistry by S.A.Iqbal and Y.Mido.

3) Encyclopaedia of Food Chemistry by Laurence Melton, Fereidoon Shahidi, Peter Valis.

4) Food Chemistry by Lillian Hog Land Meyer.

Dr. Ch. Manjula

LESSON-10

MILK AND FLESHY FOODS

10.0 OBJECTIVES:

After going through this lesson students will understand:

- Types of milk
- Various proteins present in milk and fleshy foods

STRUCTURE:

10.1 INTRODUCTION:

Proteins present in milk and fleshy foods play a crucial role in human nutrition, contributing to muscle growth, tissue repair, enzyme production, and overall body function. Milk proteins, primarily casein and whey, are high-quality proteins that provide all essential amino acids. Casein, which makes up about 80% of milk protein, is a slow-digesting protein that aids in prolonged muscle protein synthesis and calcium absorption. Whey protein, on the other hand, is rapidly absorbed, making it an excellent choice for post-workout recovery and immune support. Milk also contains bioactive peptides with potential benefits for blood pressure regulation, immunity, and gut health.

Fleshy foods, including meat, poultry, and fish, are rich sources of high biological value proteins, meaning they contain all essential amino acids in the right proportions for human needs. These proteins support muscle maintenance, hormone production, and enzyme activity. Meat proteins, such as myoglobin and actin, contribute to muscle structure and oxygen transport, while collagen and elastin provide structural support to connective tissues. Poultry and fish proteins are often leaner and provide essential nutrients like omega-3 fatty acids, which are beneficial for heart and brain health. The proteins in milk and fleshy foods are vital components of a balanced diet, offering diverse nutritional benefits that promote overall well-being and physiological functions.

10.2 MILK:

Milk is a highly nutritious natural fluid that serves as a primary source of nourishment, especially for infants. One of its most important components is protein, which plays a crucial role in growth, development, and overall health. Milk proteins are considered complete proteins because they contain all the essential amino acids required by the human body.

Milk proteins are classified into two main types: casein proteins (80%) and whey proteins (20%), each with distinct nutritional and functional properties. These proteins contribute to muscle growth, immune support, and various metabolic functions, making milk a valuable dietary component.

In addition to their biological benefits, milk proteins are widely used in the food, sports nutrition, and pharmaceutical industries. Their ability to form gels, emulsions, and foams makes them essential in dairy and processed food products.

This introduction highlights the significance of milk proteins and their role in human nutrition, health, and industrial applications.

10.2.1. Types of Milk Proteins:

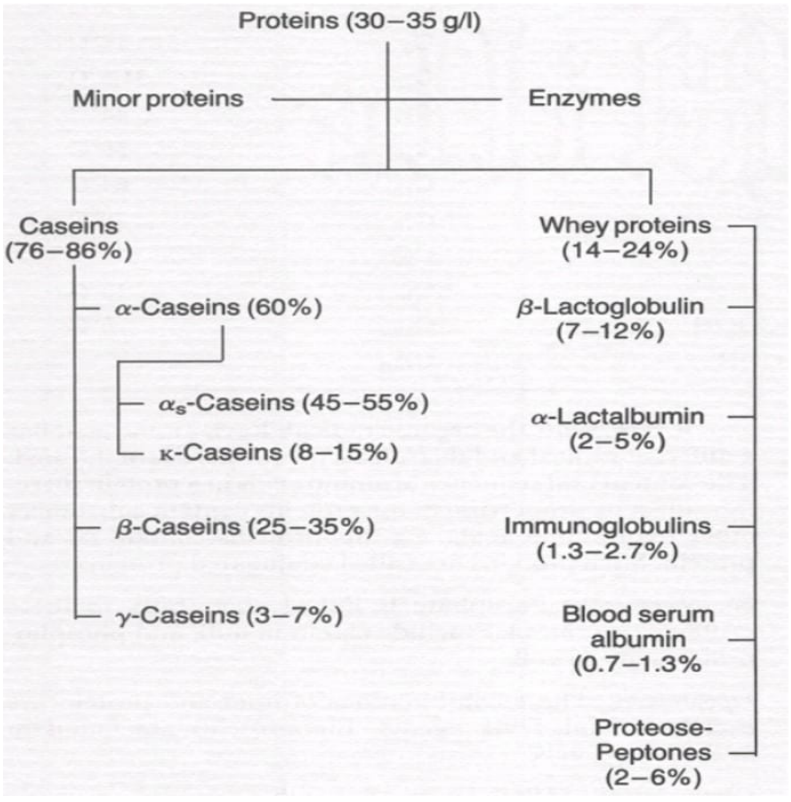

Fig: 10.2.1.a. Types of Milk Proteins

Milk proteins are classified into two major groups: Casein proteins (80%) and Whey proteins (20%). Each group has distinct structural, nutritional, and functional properties, making them essential for various biological and industrial applications.

1. Casein Proteins (80%):

Casein is the dominant protein in milk, accounting for about 80% of total milk protein. It exists in the form of micelles, which help transport calcium and phosphorus, essential for bone health. Casein proteins are slow-digesting, providing a steady release of amino acids, making them ideal for muscle maintenance and overnight recovery.

Types of Casein Proteins:

1. α-Casein (Alpha Casein):

- The most abundant casein protein.
- Helps in calcium and phosphate transport.
- Contains bioactive peptides with potential antimicrobial properties.

2. β-Casein (Beta Casein):

- Exists in different genetic variants, including A1 and A2 beta-casein.
- A2 beta-casein is easier to digest and may cause fewer digestive issues.
- Helps in emulsification and digestion.

3. κ-Casein (Kappa Casein):

- Helps stabilize casein micelles in milk, preventing early precipitation.
- Plays a crucial role in the cheese-making process.

4. γ-Casein (Gamma Casein):

- A breakdown product of β-casein.
- Has potential immunomodulatory effects.

2. Whey Proteins (20%):

Whey proteins are soluble proteins that remain in the liquid portion of milk after casein is removed (such as in cheese-making). They are fast-digesting proteins, making them ideal for muscle recovery and immune support. Whey proteins are rich in branched-chain amino acids (BCAAs) like leucine, which play a vital role in muscle growth and repair.

Types of Whey Proteins:

1. β-Lactoglobulin (Beta-Lactoglobulin) (50-60% of Whey Protein):

- The most abundant whey protein.
- Functions as a nutrient carrier (binds to vitamins and minerals).
- Can cause allergies in some individuals.

2. α-Lactalbumin (Alpha-Lactalbumin) (20-25% of Whey Protein):

- Rich in essential amino acids, including tryptophan, which promotes sleep and relaxation.
- Found in high amounts in human breast milk, making it important for infant nutrition.

3. Immunoglobulins (IgG, IgA, IgM) (10-15% of Whey Protein):

- Contribute to immune system function.
- Provide defence against infections.

4. Lactoferrin:

- Has antimicrobial, antiviral, and anti-inflammatory properties.
- Helps in iron absorption.

5. Bovine Serum Albumin (BSA):

- A minor whey protein with antioxidant properties.
- Helps transport fatty acids in the body.

10.3 PROTEINS IN FLESHY FOODS:

Fleshy foods, including meat, poultry, fish, and seafood, are rich sources of high-quality proteins that provide essential amino acids necessary for growth, muscle maintenance, and overall health. These proteins are complete proteins, meaning they contain all nine essential amino acids required by the body.

Types of Proteins in Fleshy Foods:

1. Myofibrillar Proteins (Muscle Proteins):

- Found in the skeletal muscles of animals.
- Includes actin, myosin, tropomyosin, and troponin, which are responsible for muscle contraction and movement.
- High in leucine, which supports muscle repair and growth.
- Extracted and used in food processing (e.g., meat emulsions, sausages).

2. Sarcoplasmic Proteins (Metabolic Proteins):

- Water-soluble proteins involved in energy metabolism.
- Includes myoglobin, which gives meat its red color and helps transport oxygen in muscles.
- Important for enzymatic reactions in muscle tissues.

3. Connective Tissue Proteins (Structural Proteins):

- Found in tendons, ligaments, and muscle connective tissue.
- Includes collagen and elastin, which provide structure and strength to muscles and joints.
- Collagen is used in gelatin production and bone broth for joint health.

Protein Content in Different Fleshy Foods:

Nutritional and Functional Benefits of Fleshy Proteins:

- Muscle Growth & Repair-Rich in BCAAs (leucine, isoleucine, valine), crucial for muscle synthesis.
- Immune System Support-Provides essential amino acids that strengthen immunity.
- Satiety & Weight Management-Protein-rich foods promote fullness and reduce cravings.
- Bone & Joint Health-Collagen supports cartilage and connective tissue.
- Metabolic Function-Meat proteins contain enzymes and iron, aiding energy production.

Major connective tissue proteins include:

Collagen-The most abundant connective tissue protein, responsible for toughness in meat. It converts to gelatin when cooked slowly, making meat tender.

Elastin-Provides elasticity; found in ligaments and arteries. Unlike collagen, elastin does not break down easily during cooking.

Reticulin-Supports collagen structure, forming fine connective tissue networks.

These proteins determine meat tenderness and are significant in slow-cooked dishes and bone broths.

2. Protein Content in Different Types of Meat:

Poultry (Chicken, Turkey): High in protein, low in fat (especially without skin), making it ideal for lean muscle growth.

Red Meat (Beef, Pork, Lamb): Rich in protein but higher in fat; also a good source of iron and vitamin B12.

Fish & Seafood: Good source of protein, often rich in omega-3 fatty acids, which benefit heart health.

Eggs: Contain high-quality protein and essential fats, making them a balanced protein source.

10.4 SUMMARY:

Milk and fresh meat proteins are high-quality sources of essential amino acids, playing a vital role in human nutrition. Milk proteins, primarily casein and whey, are highly digestible and contribute to muscle growth, immune function, and calcium absorption. Casein provides a slow-release protein beneficial for prolonged muscle maintenance, while whey is rapidly absorbed, making it ideal for post-exercise recovery. Additionally, milk proteins contain bioactive peptides that support heart and gut health.

Fresh meat proteins, found in beef, poultry, and fish, are complete proteins that aid in muscle development, enzyme production, and tissue repair. Meat proteins like myoglobin, actin, and collagen contribute to muscle structure, oxygen transport, and connective tissue strength. Poultry and fish proteins, in particular, offer lean protein sources rich in essential nutrients like omega-3 fatty acids, supporting heart and brain health. Together, milk and fresh meat proteins provide essential nutrients that contribute to overall well-being, making them important components of a balanced diet.

10.5 TECHNICAL TERMS:

Derived Milk-Casein, Whey, Colustrum, Meat- Collagen, Elastin

10.6 SELF ASSESSMENT QUESTIONS:

1) What are the various types of milk proteins and their significance
2) Write in detail about proteins in flesh foods

10.7 REFERENCE BOOKS:

1) Applied Food Protein Chemistry Edited by Zeynep Ustunol.
2) Food Chemistry by S.A. Iqbal and Y. Mido.
3) Encyclopedia of Food Chemistry by Laurence Melton, Fereidoon Shahidi, Peter Varli’s.
4) Food Chemistry by Lillian Hogland Meyer.

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LESSON-11

PROPERTIES OF DIFFERENT PROTEINS

11.0 OBJECTIVES:

After going through this lesson you will learn:

- Structure of proteins
- Properties of different proteins

STRUCTURE:

11.1 INTRODUCTION:

The structure of a protein depends on its amino acid sequence and local, low energy chemical bonds between atoms in both the polypeptide backbone and amino acid side chains protein structure plays a key role in the function .If a protein loses its shape at any structural level, it may no longer be functional.

11.2 STRUCTURE OF PROTEINS:

There are four levels of organization in structure of proteins: primary, secondary, tertiary and quaternary.

11.2.1 Primary Structure of Protein:

The primary structure of a protein is the linear sequence of the side chains that are connected to the protein back bone. A peptide bond is formed by the condensation of two amino acids under elimination of water.

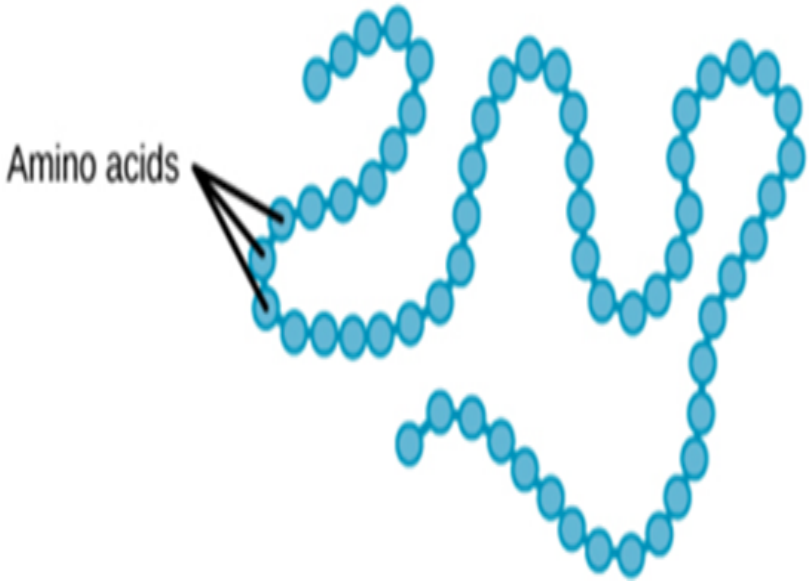

Fig. 11.2.1 (Primary structure of protein)

The amino acids are linked through the carbonyl and amino groups to produce the primary structure of the protein. The bond present between two amino acid is termed as a peptide bond. Depending on the number of amino acids forming a chain, the peptides may be termed as a dipeptide (2 amino acid units), a tripeptide (3amino acid units), and so on. If a peptide is made up of more than 10 amino acids, it is called polypeptide.

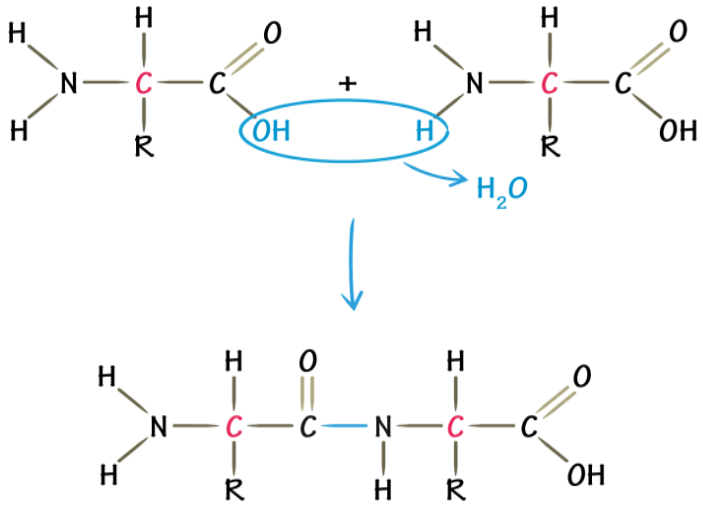

Fig - 11.2.1(Peptide bond formation)

11.2.2 Secondary Structure:

The secondary structure of a protein describes the spatial arrangement of atom in the protein back bone. Secondary structure refers to the helical nature of proteins. Formation of hydrogen bond between amino acid residues which are close to one another leads to the folding of a polypeptide chain into a helix. The secondary structure is classified into the helical structure, β pleated sheets and β turns.

Helical Structure:

The helical structure is formed by the formation of hydrogen bonds between peptide groups within the same polypeptide chain. Right handed α-helix is the common form of coiling. The α-helical structure was proposed by Pauling and Core.

Properties of α-helix:

The shape of the α- helix is maintained by hydrogen bonds. The helical chain has screw type symmetry. A hydrogen bond is formed between the peptide groups (CO and NH) of every first and fourth amino acid residues.

β pleated sheet:

The polypeptide chains arranged side by side to form sheets of molecules in some proteins called β pleated sheets. It is composed of multiple, straight segments within one polypeptide. The structure is stabilized by hydrogen bond between peptide oxygen and amide hydrogens on neighbouring segments.

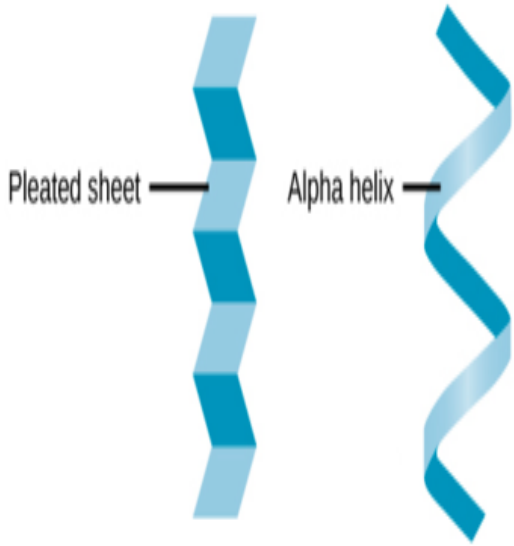

Fig. 11.2.2 (Alpha helix and β pleated sheet)

β turns:

A β turn also known as β bend is a structure that allows sharp turns in the middle of a polypeptide. A β turn is made up of four amino acid residues. The amino acids glycine and proline are often present in β turns. As glycine is small and proline is rigid, they can naturally form this sharp turn.

11.2.3 Tertiary structure:

The bending and folding of the protein in to specific three dimensional shape refers the tertiary structure. In tertiary structure the polypeptide chains become functional. Under physiological conditions, the hydrophobic side chains of neutral, nonpolar amino acids such

as phenylalanine or isoleucine may be located on the interior of the protein molecule thus protecting them from the aqueous medium. The aromatic groups of phenylalanine and tyrosineare stack together and the alkyl groups of alanine, valine, leucine and isoleucine frequently form between one another. Acidic or basic amino acid side chains are hydrophilic in nature, will generally be exposed on the surface of the protein.

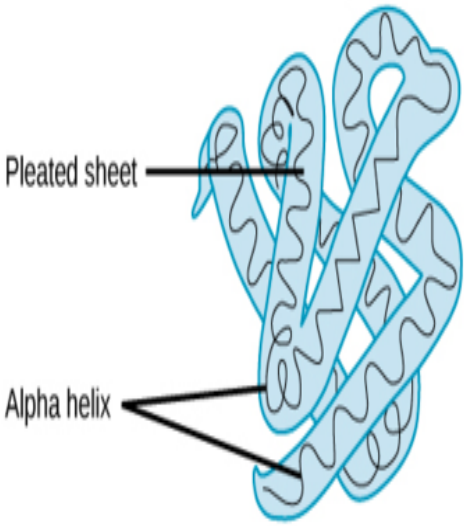

Fig. 11.2.3 (Tertiary structure of protein)

11.2.4 Quaternary structure:

The proteins contain two or more polypeptide chains which are held together by non covalent bonds are called quaternary structure. Each of the polypeptide subunits has its own primary, secondary and tertiary structure

The quaternary structure is of 2 types depending upon the nature of polypeptide chains

1) Homogeneous quaternary structure: The polypeptide chains are identical.

   Eg: Lactate dehydrogenase

2) Heterogeneous quaternary structure: The polypeptide chains are nonidentical.

   Eg: Haemoglobin

Fig. 11.2.4 (Quaternary structure of protein)

11.3 PROPERTIES OF DIFFERENT PROTEINS:

Proteins are high molecular weight compounds that yield amino acids as their principle hydrolysis products. Proteins are large molecules with some reactive groups, such as carboxyl and amino, on their surfaces. Because of the size of the molecules, they are unable to form true solutions in water. When solubility of protein is mentioned, dispersibility is proposed. Dispersion of a protein in water has the properties of a colloidal dispersion rather than a true solution.

Proteins in molecules not only associate with small molecules but also with another, sometimes to form tightly bound and sometimes loosely bound products. Proteins molecules are quite sensitive to many reagents and conditions. They may readily undergo small transforms in structure. Without any alteration in the protein molecule, it is very difficult to isolate a protein from its natural source, which occurs in a cell or biological fluid.

The shape of protein molecules varies greatly, Some exist as fiber, others as spheres while intermediate are many shaped like spindles, cigars, etc. The chain must be folded or coiled in some fashion and there must be some bonds which hold the chain a relatively permanent shape.

11.3.1 Physical properties of proteins:

Colour and taste:

Proteins are colour less and usually tasteless. These are homogeneous and crystalline.

Shape and size:

The proteins range in shape from simple crystalloid spherical structures to long fibrilluar structures. Two distant patterns of shapes are

Globular proteins: These are spherical in shape and occur mainly in plants (seeds and leaf cells).These are bundles formed by folding and crumpling of protein chains. Eg: pepsin, insulin, ribonuclease etc.

Fibrillar proteins: These are thread like or ellipsoidal in shape and occur generally in animal muscles. Eg: fibrinogen, myosin etc.

Molecular weight:

The proteins generally have large molecules weights. It might be noted that the values of molecular weights of many proteins are close to or multiples of 35kDa and 70kDa.

Colloidal nature:

Because of their giant size,the proteins exhibit many colloidal properties .Their diffusion rates are extremely slow and they may produce considerable light scattering in solution ,thus resulting in visible turbidity (Tyndall effect).

Denaturation:

Denaturation refers to the changes in the properties of a protein. The denatured proteins losses its biologic activity. In many cases the process of denaturation is followed by coagulation-a process where denatured protein molecules tend to form large aggregates and to precipitate from solution.

Solubility:

The solubility of proteins are influenced by pH. Solubility is lowest at its isoelectric point and increases with increasing acidity or alkalinity. This is because when the protein molecules exist as either cations or anions, repulsive forces between ions are high, since all the molecules possess excess charges of the same sign. Thus, excess charges of the same sign, they will be more soluble than in the isoelectric state.

Optical Activity:

Proteins are optically active. They are usually levorotatory. They rotate the plane of polarization to the left, when polarized light of wavelengths in the visible range is used. The specific rotation of most L-amino acids varies from -30° to+30°. The optical rotation of a protein depends on all of the amino acids of which it is composed.

Amphoterism of proteins:

It is the ability of proteins to react either as acids or as bases. The free carboxyl groups of proteins are weekly ionized but are available for reaction with bases. The free amino groups are hydrogen acceptors and are available for reaction with acids.

Binding of ions:

Proteins can bind both cations and anions through reaction with either the carboxyl group or the free amino groups. At pH values above the isoelectric point, a protein exists as negative ion and binds or reacts with cations. At pH values below the isoelectric point it will exist as a positive ion and will react with or bind anions. The precipitation of protein by heavy metal ions depends on the formation of these protein salts.

Hydration of Proteins:

Proteins can form hydrates with water and this type of reaction is important in food chemistry. A protein molecule contains a number of groups in which a nitrogen or an oxygen atom contains a pair of unshared electrons and is therefore capable of forming a hydrogen bond.

The nitrogen in the peptide link as well as the nitrogen in the free amino group are in this condition, and by their relative negativity can attract the hydrogen of a molecule of water. The double bonded oxygen of the carboxyl (-COOH) or carbonyl (CO) group of the peptide link is more strongly negative and has a greater attraction for the hydrogen than the nitrogen. The water molecule which has been bound can attract another molecule of water

since it possesses oxygen with an unshared pair of electrons. Aggregates of water can buildup around each polar group on the protein molecule. Electrolytes, sugars, alcohols and many other substances have this tendency to combine with water and from hydrates and may compete with protein for the water.

11.3.2 Functional Properties of Proteins:

Proteins provide a wide range of functional qualities in foods, including water bindings/holding, gelation, foaming, emulsification, and more. Egg white proteins are one of the best choices to make soft gels and emulsify fats. Most of all, eggs white proteins are superior for use in applications that require a heat stable of foam like souffles and meringues. A variety of protein ingredients isolated from animal and plant sources are commercially available for use in these applications .In general, animal proteins have better functional qualities compared to plant proteins. The amino acid sequence and distribution of polar and nonpolar residues are critical to most functional properties. Extrinsic factors affecting protein functionality are temperature, pH and salt.

Hydration is most important determinant of food protein functionally quality. This is the ability to interact with water. Proteins with poor water compatibility do not work well in applications requiring solubility or dispersibility. A protein's affinity for water depends upon its amino acid composition. Specifically, those amino acids with polar or polar non ionized R groups. Dry protein ingredients typically contain 5% to 10% water that is bound in a very thin layer to polar amino acid R groups. This class of water referred to as bound water.

Bound water is defined as water that cannot be removed by conventional drying methods. Approximately 6-7 water molecules are bound to polar R groups of amino acids those include aspartic, glutamic, arginine, histidine and lysine. Additional 2 to 3 water molecules are bound to polar non ionized amino acids like arginine, glutamine, serine, threonine, and tyrosine. Bound water significantly aids in rehydration of proteins.

Commercial protein ingredients like milk casein, whey protein isolate and soy protein isolate have water isolate have water binding capacities ranging from 0.3 to 0.9 g of water per gram of protein. Milk caseins contain 6-7 polar phosphate groups on each molecule.

Each phosphate group has a strong negative charge that attracts water molecules and contributes to the overall water binding capacity of casein. The water holding capacity of meat is due to the nature of its proteins and the structure of the myofibril organelle.

Myofibrils are composed of thick and thin filaments that slide past each other during contraction and relaxation. Myofibrils are a key factor in this water holding capacity because their open structure facilitates trapping water by capillary action. The final pH of meat and the rate at which it declines postmortem strongly influences its water holding capacity.

11.4 SUMMARY:

The structure of a protein depends on its amino acid sequence and local, low energy chemical bonds between atoms in both the polypeptide backbone and in amino acid side chains. There are four levels of organization in structure of proteins: primary, secondary, tertiary and quaternary. Proteins are large molecules with some reactive groups, such as carboxyl and amino, on their surfaces. The shape of protein molecules varies greatly. Some exist as fibers, others as spheres while intermediate are many shaped like spindles, cigars etc. Proteins provide a wide range of functional qualities including water binding/holding, gelation, foaming, emulsification, and more. Denaturation is one of important physical property of the proteins. Denaturation refers to the changes in the properties of a protein. Due to denaturation, loss of their biological activity and protein molecules tend to form large aggregates and to precipitate from solution.

11.5 TECHNICAL TERMS:

Peptide bond, α helix, β pleated sheets, Quaternary structure, Optical activity.

11.6 SELF ASSESSMENT QUESTIONS:

1) Explain in detail about the structural organization of proteins?
2) Write an account on physical properties of proteins?
3) Discuss about the functional properties of proteins?

11.7 REFERENCE BOOKS:

1) Applied Food Protein Chemistry Edited by Ustunol.
2) Food chemistry by S.A. Iqbal and Y.Mido.

https://alevelbiology.co.uk/notes/proteins-physical-chemical-properties/#0physical-properties-of-proteins-

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LESSON-12

DETERMINATION OF PROTEINS BY ELECTROPHOESIS, MICRO-KJELDHAL METHOD

12.0 OBJECTIVES:

After going through this lesson you will learn:

- Electrophoresis
- Micro-Kjeldahl method.

STRUCTURE:

12.1 INTRODUCTION:

“Electrophoresis is a standard laboratory technique by which charged protein molecules are transported through a solvent by an electrical field”.

- Both proteins and nucleic acids may be separated by electrophoresis,
- Which is a simple, rapid, and sensitive analytical tool.
- Most biological molecules carry a net charge at any pH other than their isoelectric point and will migrate at a rate proportional to their charge density.

The mobility of a molecule through an electric field will depend on the following factors:

- Field strength,
- Net charge on the molecule,
- Size and shape of the molecule,
- Ionic strength, and
- Properties of the matrix through which the molecule migrates (e.g., viscosity, pore size).

Types of matrix used in protein electrophoresis:-

1. Polyacrylamide and agarose are two support matrices commonly used in electrophoresis. These matrices serve as porous media and behave like a molecular sieve.
2. Agarose has a large pore size and is suitable for separating nucleic acids and large protein complexes. Polyacrylamide has a smaller pore size and is ideal for separating majority of proteins and smaller nucleic acids.

Several forms of polyacrylamide gel electrophoresis (PAGE) exist, and each form can provide different types of information about proteins of interest.

- Denaturing and reducing sodium dodecyl sulfate PAGE (SDS-PAGE) with a discontinues buffer system is the most widely used electrophoresis technique and separates proteins primarily by mass.
- Non denaturing PAGE, also called native-PAGE, separates proteins according to their mass/charge ratio.
- Two-dimensional (2D) PAGE separates proteins by native isoelectric point in the first dimension and by mass in the second dimension.

SDS-PAGE separates proteins primarily by mass because the ionic detergent SDS denatures and binds to proteins to make them uniformly negatively charged. Thus, when a current is a applied, all SDS-bound proteins in a sample will migrate through the gel toward the positively charged electrode. Proteins with less mass travel more quickly through the gel than those with greater mass because of the sieving effect of the gel matrix. Once separated

by electrophoresis, proteins can be detected in a gel with various stains, transferred onto a membrane for detection by western blotting and/or excised and extracted for analysis by mass spectrometry.

12.2 PRINCIPLES OF ELECTROPHORESIS:

Electrophoresis is the motion of the dispersed particle under the influence of electric charge. When any charged ion or molecule placed in an electric field it migrates. The rate of movement of the charged particle depends on its net charge, size, shape and the applied current, which can be represented by the following equations:

$$V = \frac{E.q}{f}$$

Where v= velocity of migration of the molecule E=electric field in volts/cm q= the net electrical charge on the molecule

f = the frictional coefficient which is a function of the mass and shape of the molecule

The migration of a charged molecule in an electric field is generally expressed in terms of electrophoretic mobility (μ) which is defined as the velocity per unit of electric field

$$\mu = \frac{v}{E} = \frac{E.q}{f.E} (since\ v = \frac{E.q}{F})$$

$$= \frac{q}{F}$$

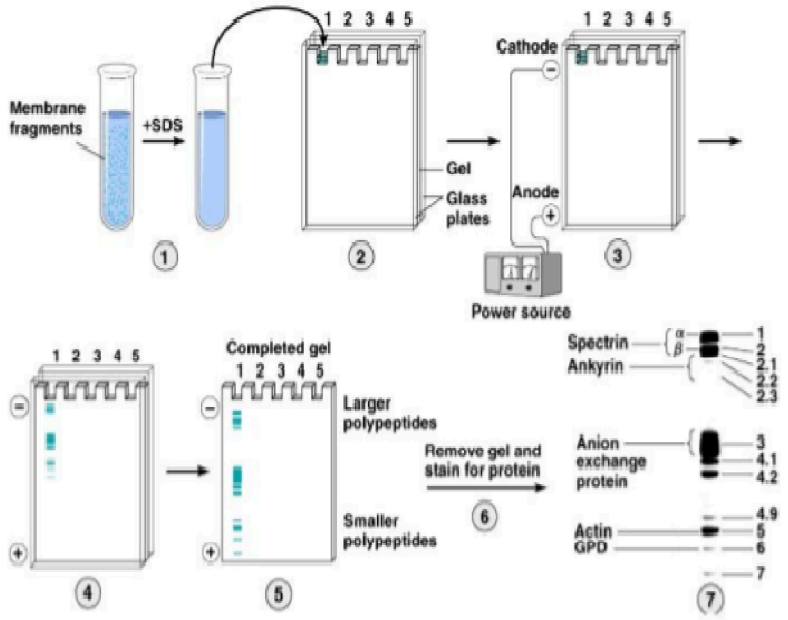


Fig. 12.1: Schematic Diagram of Process of Electrophoresis (SDS-PAGE) to Separate the Proteins

12.2.1. Paper Electrophoresis:

Paper electrophoresis is a commonly used electrophoretic technique for the analysis and separation of small molecules. Paper electrophoresis is not suitable to resolve macromolecules (e.g proteins) because the adsorption and surface tension involved with paper chromatography generally denature the protein and subsequently poor resolutions occur. In this type of electrophoresis the sample is applied as a circular point or spot on a strip of cellulose acetate paper or whatman filter paper moistened with buffer solution. The end of the spotted paper is immersed in separate reservoirs containing buffer solution and fitted electrodes. When electric current passes through the electrodes the ions present in the sample migrates at different rate towards oppositely charged electrodes at characteristics rate.

12.2.2. Gel Electrophoresis:

In gel electrophoresis the aqueous buffers, supported with polymeric gel matrix is used to separate the molecules. Gel electrophoresis has a number of advantages .Gel electrophoresis can accommodate larger samples than most of the paper electrophoresis and so can be used for preparative scale electrophoresis of macromolecules. One of the major advantage of the gel electrophoresis is that it can. Many gel like agents are used in gel electrophoresis.

12.2.2.1. Starch Gel Electrophoresis:

Starch gel, formed by heating partially hydrolyzed starch in a buffer, is used to separate molecules based on both charge and size. The gel's sieving effect means that for molecules with similar charges, smaller molecules will move through the gel faster and be eluted first. This technique is particularly useful for separating isozymes.

12.2.3. Polyacrylamide Gel Electrophoresis:

Polyacrylamide gel is the most useful and widely used techniques for the separation and characterization of nucleic acids and proteins.

Polyacrylamide gel have definite advantages like : it is chemically inert, having superior resolution,stable over a wide range of pH, wide range of gel can be prepared by using polyacrylamide gel and having good temperature and ionic strength. Polyacrylamide gel is prepared through polymerization of monomer of acrylamide and N, N’-methylene - bis-acrylamide (Fig.). Polyacrylamide gel can be prepared by using either chemical or photochemical free radical sources to initiate polymerization process. Chemical method is commonly used techniques for the polymerization of polyacrylamide gels where the free radical initiator, ammonium persulfate (APS), is added along with a N,N,N’,N’-trimethylethylenediamene (TEMED) catalysts. In the presence of monomer, cross linker and appropriate buffer the APS and TEMED generate the free radicals to induce the polymerization. However in photochemical method a photosensitive compound replace the ammonium sulphate and generate free radicals when irradiated by UV light.

The pore size of the polyacrylamide gel can be modified by changing the concentration of acrylamide gel. For larger pore size the amount of monomer can be decreased and for smaller pore size gel the concentration of monomer or cross linker can be increased.

In PAGE electrophoresis the separation depends on the friction of the protein within the matrix and the charge of the given protein as given in the formula:

$$\text{Mobility} = \frac{(Voltage\ \ applied\ )x(total\ \ charge\ \ on\ the\ protein\ )}{Fricti\ on\ of\ \ the\ molecule}$$

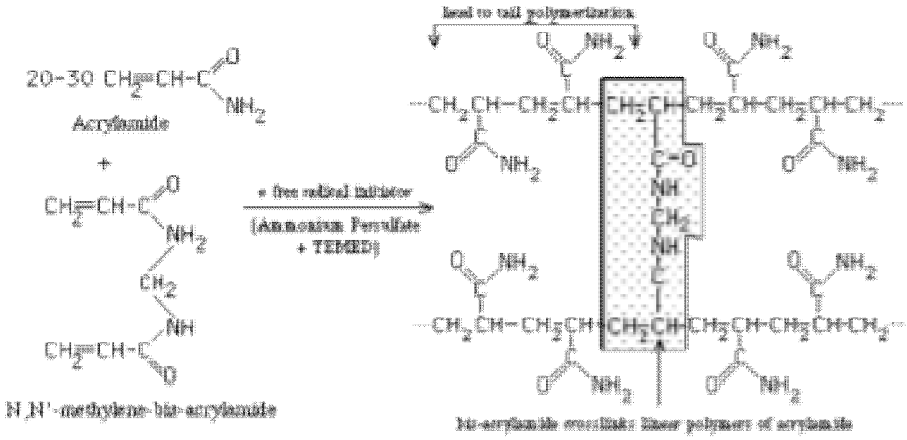


Fig. 12.2: Image for the Formation of Polyacrylamide gel through the Polymerization

12.2.3.1. Native Discontinuous Polyacrylamide Gel Electrophoresis:

In discontinuous gel electrophoresis buffers ions used in the gel and in the electrode reservoir are different and it contains two types of gels i.e. large pore stacking gel and small pore gel. Large stacking gels are polymerized on small pore resolving gel. The buffers used for these two gels of having different ionic strength and pH. In discontinuous native gel electrophoresis relatively large volumes of dilute solutions of protein sample can be loaded in comparison with native discontinuous gel electrophoresis.

12.2.3.2. SDS-Polyacrylamide Gel Electrophoresis (Non Reducing):

In this type of electrophoresis the proteins are treated with anionic detergent such as sodium dodecyl sulphate or sodium lauryl sulphate to dissociate into their constituents subunits. The protein is denatures by heating at 100c in presence of excess SDS. During reaction most polypeptide binds the SDS in constant weight ratio. Since the charges of protein are insignificant in respect of negative charges of SDS, so the charges of the SDS polypeptide complexes are almost same hence the migration of the protein during electrophoresis is strictly depends on the size of the polypeptide. This technique is not only suitable for the analysis of the composition of the polypeptides but also suitable for the determination of the molecular weight of the given polypeptide.

12.2.2.2. SDS-Polyacrylamide Gel Electrophoresis (Reducing):

In this method the sodium dodecyl sulfate (SDS) is used to determine size and number of protein chains in the protein preparation. The prepared protein is treated with an excess of SDS and soluble thiol. The thiol reduces all disulphide bonds of proteins and SDS binds to all regions of the proteins and disrupts most non-covalent inter and intramolecular protein interaction. The combined effect of thiol and SDS denatured the protein and yields unfolded, highly anionic (negatively charged) polypeptide chains. The treated polypeptide chains are then resolved by using SDS saturated Polyacrylamide gel electrophoresis. The excess of SDS is used to maintain the denatured conditions of the protein throughout the electrophoretic separation. Due to the treatment of the SDS the charge to the mass ratio of the proteins in the samples approximately the same and so the migration is solely depends on the size or molecular weight of the polypeptides. So in this electrophoresis the larger particle will experience greater friction during migration and will migrate slowly than smaller polypeptides. The schematic representation of SDS-PAGE electrophoresis (reducing) are given below.

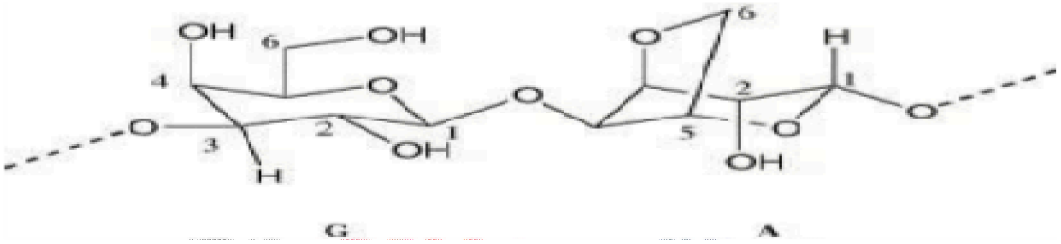


Fig. 12.3: Basic Diasaccharide Repeating Units of Agrose

12.3. AGAROSE GEL ELECTROPHORESIS:

Agarose gel electrophoresis is the commonly used techniques for the analysis of high molecular weight nucleic acid i.e. DNA and RNA. Agarose gel is more porous and has a larger pore size. Agarose is a linear polymer of galactose and 3,6 anhydrogalactose. The polymer of agarose may contain upto 100 monomeric unit, with an average weight of 10,000 Dalton. For the preparation of agarose gel, the agarose are boiled in an aqueous buffer (either Tris acetate or Tris borate) containing EDTA and then poured into a mold and allowed to harden. The pore size of the agarose gel can be controlled by changing the percentage of agarose in the dissolved solution. Higher the agarose concentration, smaller the pore size and lower the agarose concentration, larger the pore size. When an electric field is applied across the DNA loaded sample, The DNA molecules (negatively charged at neutral pH) migrates towards oppositely charged electrodes. The rate of migration of DNA depends on their molecular size and confirmation. The charge / mass ratio in the nucleic acid is one so rate of migration of DNA molecule is inversely proportional to log10 of their molecular weight.

Smaller molecules of DNA will travel faster as compared to larger ones. Confirmations of DNA also affect the rate of migration of DNA and so DNA molecule of having same molecular weight with different confirmation will migrate at different rates. The order of migration velocity of DNA is: supercoiled DNA> linear double stranded DNA> open circular DNA. Tris acetate (TAE), Tris borate (TBE) or Tris phosphate (TPE) is the commonly used buffers in agarose gel electrophoresis.

A protein which is in a pH range below the isoelectric point has the positive charge so it migrates towards negative charge. As it migrates towards gradients of increasing pH so the overall charges of the protein decreases and reaches to the pH region of its isoelectric point. At the isoelectric pH the net charges becomes zero and so migration ceases and proteins becomes focused into sharp stationary bands.

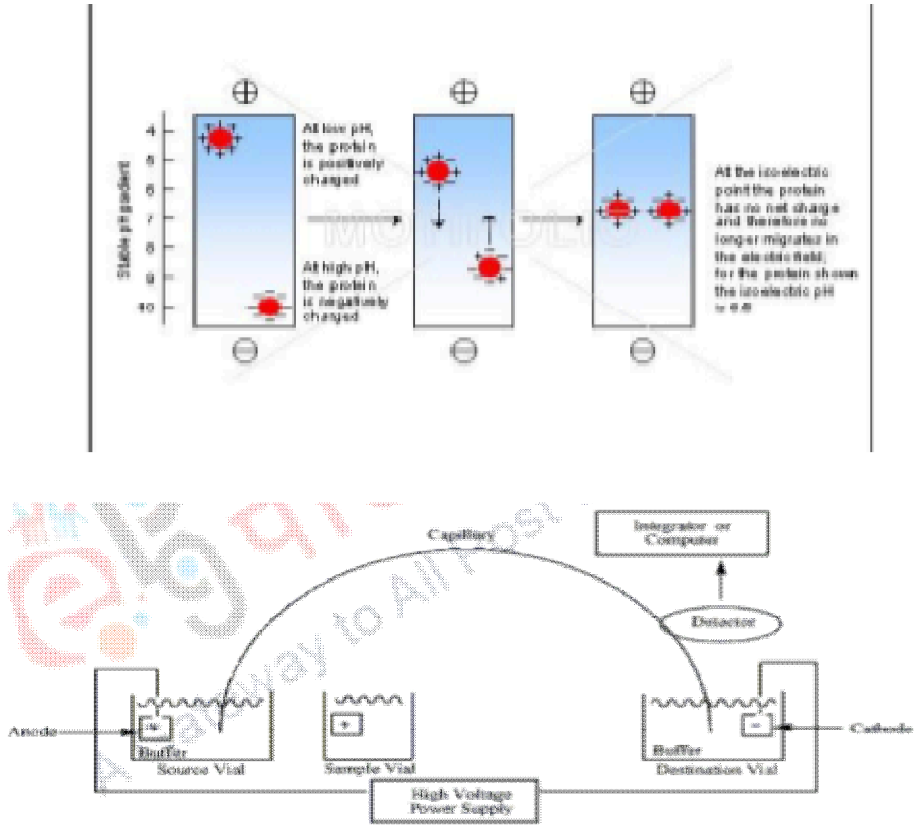


Fig. 12.4: Schematic Representation of the Separation of Protein molecule by Isoelectric Focusing Electrophoresis & Capillary Electrophoresis

12.4 IMMUNOELECTROPHORESIS:

Immuno-electrophoresis is the one of the types of agarose gel electrophoresis where the separation of the proteins depends on their charge to mass ratio and their antigenicity. The given protein is separated by using gel electrophoresis and is then allowed to interact with specific antibody preparation placed in through cut placed the centre of the gel. The antibody

diffuses through the gel and interacts with the electrophoresed proteins for which the antibody has affinity and gives precipitate. This technique is suitable for the identification of the number of antigens in the sample and also indicates the relative amount of immuno-reactive material.

12.5 MICRO-KJELDAHL METHOD:

Micro- Kjeldhal Method:

The Micro Kjeldahl method is a laboratory technique used to determine the nitrogen content in various types of samples, such as food, soil, or fertilizers. This method is a scaled-down version of the classical Kjeldahl method, making it suitable for smaller sample sizes.

Principle:

The Micro Kjeldahl Method is based on the conversion of organic nitrogen into ammonium sulfate by digestion with concentrated sulfuric acid (H_2SO_4) in the presence of a catalyst. The ammonium ions (NH4+) formed are then converted to ammonia (NH_3) by adding a strong alkali (sodium hydroxide, NaOH). The ammonia is distilled and absorbed in boric acid (H_3BO_3), then titrated with a standard acid (HCl or H_2SO_4) to determine nitrogen content.

Steps of the Micro Kjeldahl Method:

1) Digestion:

- The sample is digested in a flask using concentrated sulfuric acid (H_2SO_4) to break down the organic matter.
- A catalyst (usually copper sulfate or potassium sulfate) and a small amount of selenium or mercury are added to accelerate the reaction.
- The digestion converts nitrogen in the sample into ammonium sulfate $(NH_4)_2SO_4$

Digestion (Conversion of Organic Nitrogen to Ammonium Sulfate)

$$(\text{Protein}) + H_2SO_4 \xrightarrow{\text{heat}} (NH_4)_2SO_4$$

The organic nitrogen is converted into ammonium sulfate.

- The carbon and hydrogen in the sample are oxidized to CO_2 and H_2O.

2) Neutralization:

- The digest is neutralized by adding an alkaline solution (usually sodium hydroxide, NaOH), which converts ammonium ions (NH_4^+) into ammonia gas (NH_3).
- The reaction produces a basic environment for ammonia release.

Neutralization $(NH_4)_2SO_4 + 2NaOH \rightarrow 2NH_3 + Na_2SO_4 + 2H_2O$

- Sodium hydroxide (NaOH) is added to neutralize sulfuric acid.

3) Distillation:

- The liberated ammonia (NH_3) is distilled and absorbed in a solution of boric acid (H_3BO_3).

Ammonia Release

- Ammonia (NH_3) gas is released.

$$NH_3 + H_3BO_3 \rightarrow NH_4^+ + H_2BO_3^-$$

4) Titration:

- The captured ammonia is titrated with a standard acid solution (like HCl or H_2SO_4) to determine the amount of nitrogen in the sample.

$$H_2BO_3^- + HCl \rightarrow H_3BO_3 + Cl^-$$

Calculation:

Nitrogen to Protein Conversion

- Nitrogen percentage is determined from titration.
- Protein content is calculated using the factor 6.25
- Nitrogen content is calculated and converted to protein content using the protein conversion factor (usually 6.25 for general proteins).

$$\%N = \frac{\text{Corrected Acid Volume} \times \text{Normality of Acid} \times 14 \times 100}{\text{Sample weight}}$$

Applications:

- Determining protein content in food (since protein content is calculated from nitrogen content)
- Soil fertility analysis
- Testing in fertilizers and animal feeds

Advantages:

- Highly accurate for nitrogen determination
- Works with small sample sizes
- Suitable for complex organic samples

Disadvantages:

- Time-consuming

- Involves hazardous chemicals
- Requires skilled personnel to perform accurately

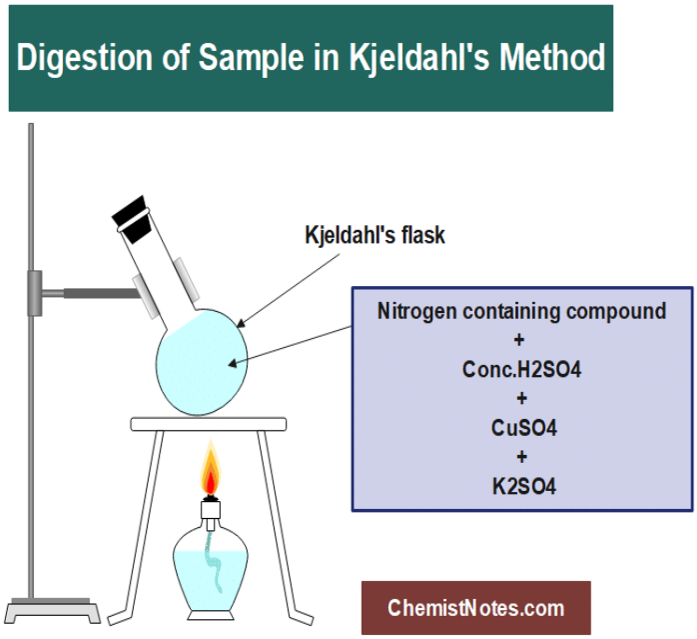


Fig. 12.5: Kjeldahl's Method

Dumas Method:

The Dumas method is an alternative to the Kjeldahl method for determining nitrogen content in organic compounds. It is faster, automated, and does not require the use of hazardous chemicals such as sulfuric acid. It is often employed in laboratories for food, feed, and environmental analysis.

Principle:

The Dumas method involves complete combustion of the sample in the presence of oxygen at high temperatures, converting all nitrogen in the sample to nitrogen gas (N_2). The nitrogen gas is then quantified using gas chromatography or thermal conductivity detectors.

Steps Involved:

1) Sample Combustion:

The sample is combusted at high temperatures (around 900°C–1100°C) in the presence of oxygen. Nitrogen in the sample is converted to nitrogen gas (N_2), along with CO_2, H_2O, and other by-products.

2) Removal of By-products:

The combustion gases pass through filters to remove carbon dioxide and water.

3) Detection:

The remaining nitrogen gas is measured using a thermal conductivity detector (TCD).

4) Calculation of Nitrogen Content:

The amount of nitrogen gas detected is used to calculate the nitrogen content in the sample.

Applications:

- Food and feed analysis: Protein content determination
- Agriculture: Fertilizer analysis
- Pharmaceuticals: Analysis of nitrogen-containing compounds
- Environmental monitoring: Soil and waste analysis

Advantages:

- Faster and more automated than the Kjeldahl method
- No hazardous chemicals are required
- Suitable for large sample throughput
- Accurate and precise results

Disadvantages:

- More expensive due to specialized equipment
- Not as widely available in smaller laboratories
- Requires a pure oxygen supply and maintenance of combustion chambers.

12.6 SUMMARY:

Electrophoresis is a laboratory technique used to separate charged molecules, like proteins or DNA, based on their size and charge by applying an electric field, causing them to migrate through a gel medium, while the Kjeldahl method is a chemical analysis technique used to determine the total nitrogen content in a sample, often used to estimate protein levels, by converting organic nitrogen into ammonia through digestion with sulfuric acid and then quantifying the ammonia through titration; essentially, electrophoresis separates molecules based on their charge while Kjeldahl measures the total nitrogen content in a sample.

12.7 TECHNICAL TERMS:

Lysine, Histidine, Capillary electrophoresis, Tris acetate (TAE),Tris borate (TBE) or Tris phosphate (TPE).

12.8 SELF ASSESSMENT QUESTIONS:

1) Explain about the process of kjeldahl's method in detail.
2) Write about the Electrophoresis.
3) What are the different types of electrophoresis.

12.9 REFERENCE BOOKS:

1) Applied Food Protein Chemistry edited by Ustunol.

2) Food Chemistry by S.A.Iqbal and Y.Mido.

3) https://alevelbiology.co.uk/notes/proteins-physical-chemical-properties/#0---physical-properties-of-proteins--

Dr. Ch. Manjula

LESSON-13

FRUITS AND VEGETABLES

13.0 OBJECTIVES:

After going through this lesson students will understand:

- Chemistry of fruits and vegetables
- Post harvesting changes during storage
- Composition of fruits and vegetables

STRUCTURE:

13.1 INTRODUCTION:

Fruits and vegetables share numerous similarities in their composition, cultivation, harvesting, storage, and processing. Many vegetables, like tomatoes and cucumbers, are technically fruits from a botanical standpoint, as they contain seeds. However, the term "vegetable" is more commonly used for plants eaten as part of the main meal, while "fruit" typically refers to those consumed separately, as a dessert, or as a snack.

13.2 CHEMISTRY:

The chemical composition of fruits and vegetables vary greatly. Except nuts and dates, all are high in water with a range from approximately 70% for pears, bananas, figs, etc. to 98% for vegetable marrow. Legumes and nuts are relatively low in protein.

All fruits and vegetables contain some carbohydrates. Carbohydrates in fresh fruits are present as cellulose and pectic substances in the cell walls, but these compounds are indigestible and not available in the human body. Starch is present in almost all fruits and vegetables although it may disappear on ripening. Fructose, glucose 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.

The amount of lipids in fruits and vegetables is usually very small. Nuts are the general exception, while a few vegetables such as avocados are a rich source of fat.

Leafy vegetables 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, provitamin A, and in the B complex. The raw leaves often contain good amounts of ascorbic acid.

Flowers, buds and stems 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 have small amounts of vitamins and minerals and few have moderate amounts of ascorbic acid and riboflavin.

Bulbs, roots and tubers are high in water, moderate in cellulose and contain an appreciable amount of available carbohydrate. The available carbohydrates are starches, glucose, and some amount of sucrose. The amounts of the vitamins and minerals are not high but are valuable additions to the diet. The amount of ascorbic acid in potatoes has made the difference between gross scurvy and its absence in large number of population.

Seeds are relatively low in water and cellulose, containing a fair amount of protein and large amount of starch. They are remarkable sources of the B complex vitamins and iron.

Vegetable fruits are relatively high in water and cellulose but low in calories and protein. Many contain good amounts of vitamins and minerals. Tomatoes are notable for ascorbic acid and thiamine.

13.3 COMPOSITION:

The Fruits and vegetables contain a number of biochemical constituents. Those include sugars, acids, fats, flavouring compounds, phenols, vitamins and minerals, exhibit an important role in establishing the composition and quality of fruits and vegetables. These are the main source of nutrition.

Sugars whichever in free state play crucial role in imparting attractive colour, flavour, appearance and texture to the fruits. Falvour is basically the balance between sugars and acids and in addition, specific flavour constituents are often glucosides. The attractive colour of many fruits are due to sugar derivative, is found extensively and sometimes abundantly in fruits. Sugars are the primary substrate for respiration.

Organic acids are crucial compounds in fruits and vegetables. They contribute significantly to taste and flavor, while also serving as an energy source for respiration within plant cells. Key organic acids found in fruits include malic, citric, tartaric, quinic, succinic, and shikimic acid. Beyond their sensory roles, organic acids play vital roles in various plant processes like photosynthesis and respiration. They are essential components of these fundamental metabolic pathways. Organic acids contribute to the synthesis of phenolic compounds, lipids, and volatile aromas, which further enhance the sensory qualities of fruits and vegetables. By interacting with insoluble pectin complexes, organic acids facilitate the release of calcium, enhancing the solubility of these compounds.

At the time of ripening, loss of chlorophyll is accompanied by the biosynthesis of one or more pigments usually either anthocyanins or carotenoids which are localized within the vacuole and chloroplast, respectively. Anthocyanins can give rise to colours from red to blue and usually occur in a wide range of types in individual fruit. Carotenoids are synthesized in green tissues. β-carotene and lycopene are the major products of carotenoids. Flavour in fruits and vegetablesis depend upon the nature of volatiles includes alcohols, aldehydes, esters and many other chemical groups.

Phenolics are widely distributed in plant kingdom and make up a large class of compounds, including anthocyanins, leucoanthocyanins, anthoxanthins, hydroxybenzoic acids, glycosides, sugar esters of quinic and shikimic acids, esters of hydroxycinnamic acids and coumarin derivatives. Phenolics are important in determining colour and flavour of the fruits. It has been suggested that phenolics are originally the byproducts of the metabolism of aminoacids.

Phenolic compounds contribute to the sensory qualities of fruit (colour, astringency, bitterness and aroma) and play a vital role in resistance to attacks by pathogens and stress.

L-ascorbic acid is the naturally occurring ascorbic acid in fruits. In all the living plant cells ascorbic acid is probably present in trace amounts where it plays the function as a terminal oxidase along with cytochrome oxidase, in the electron transport system.

Proteins are present in low concentration in fruits but high in some vegetables (peas and beans). These are the components of nuclear and cytoplasmic structures which take part in determining and maintaining cellular organization, but are also involved in metabolism during growth, development, maturation and postharvest life of the fruit. Proteins are concerned with various physiological events including the synthesis of enzymes.

Macro and micro nutrients play a vital role in plant metabolism. Nitrogen deficiency leads to the reduction in fruit size as a consequence of decrease in protein. Phosphorus plays a key role in energetics of metabolism and biosynthetic reactions as a component of ATP and numerous other phosphorylated compounds. Potassium acts as activator of many enzymes. Calcium plays an important role in control the enzyme activity, respiration and ethylene production.

Magnesium content appearsto display a vital role in chlorophyll synthesis, protein synthesis, pH control, enzyme activity and energy transfer in plants. The zinc may exert profound influence on auxin metabolism.

13.4 POST HARVEST CHANGES:

Fruits and vegetables are highly perishable in nature and may be not acceptable for consumption if not handled properly following harvesting. These are passed through a long channel before their use, which may lead to a number of undesirable physico-chemical changes in their composition.

Fruits and vegetables which are also processed into variety of products include jam, juice, sauce, frozen food, canned and fermented products etc. Require essentially a right stage of picking, like for juice making the fruits should be picked at ripe and soft stage, while juicy with best flavour stage is most suitable for the preparation of wine.

Effective postharvest management of fruits and vegetables, aimed at maximizing their consumable quality, requires a strong understanding of several key factors. These include the optimal maturity and harvesting stage, proper postharvest handling techniques, and the physiological changes in composition that occur during ripening, transportation, and marketing.

After being picked, fruit continues to undergo chemical changes, eventually spoiling due to bacteria, fungi, or yeast. These changes affect the fruit's edibility and commercial value. Fruits like apples, pears, citrus fruits, and bananas are stored for varying periods before consumption, making the chemical transformations they experience economically significant. More perishable fruits, such as cherries, plums, and berries, are typically sold soon after harvest. Extending their shelf life requires preservation methods like canning or freezing.

Harvesting:

Harvesting is an important operation in horticultural crop production and any insufficiency during this time may lead to the loss of whole year’s work. Harvesting with inappropriate method leads to the damage of crop by bruising which can be caused by compression (due to overfilling of boxes or in bulky stores), impact (due to dropping of crop or from something hitting the crop) or vibration (due to loose packing during transportation). So during harvesting, factors like delicacy of crop, maturity criteria, time method of

harvesting, mode of packaging and transportation the importance of speed during and directly after harvesting, economy of the operations and the need for the harvesting method to fulfill the market requirement should be taken into considerations.

In addition to harvesting methods, the time of harvest plays a crucial role in maintaining the best quality of crop during the course of postharvest handling and storage. Several methods have been developed to determine when a crop is ready for harvest, and these methods vary depending on the specific crop.

Post-harvest fruits undergo numerous changes, including alterations in respiration, water content, carbohydrates, organic acids, and pH.

13.4. Respiration:

Apples and pears have a low respiration rate at harvest time. However, after picking, their oxygen intake and carbon dioxide production increase, peaking at a point called the climacteric. This peak is then followed by a gradual decline in respiration, known as senescence.

Bananas, typically harvested green, also exhibit a climacteric as they ripen. Tomatoes, and potentially other fruits, also demonstrate this distinct respiratory shift.

13.4.2 Water Loss:

Once fruit or vegetable is picked, it's cut off from its water supply, yet it continues to lose moisture. The fruit skin typically prevents water absorption. A key challenge in apple storage is minimizing water loss to prevent shriveling and reduced market value. Dry air, especially at high temperatures, accelerates water loss.

Rapidly cooling apples after harvest significantly reduces this loss compared to slow cooling. Interestingly, bananas experience a shift in water content during ripening, with the pulp gaining moisture and the peel losing it. Bananas are protected from water loss by their skin's waxy coating.

13.4.3 Changes in Carbohydrates:

Many changes occur in the carbohydrate fraction of fruit during ripening, climacteric and senescence conditions. Green fruit usually contains an abundance of starch, but low in the soluble sugars those give sweetness to ripe fruit.

On ripening, there is a decrease in starches and increase in sugars concentration. So it has been expected that the sugars are produced in the expense of starch. In apples and pears fructose, glucose and sucrose are most important sugars.

13.4.4 Change in Texture:

One of the most obvious changes in fruit is the alteration in texture. Since pectic substances are present in the cell walls, so that a clear picture of the changes in these substances after harvest. Apples in storage slowly soften and the rate depends on the

temperature of the storage barn as well as the variety of the apple. A decrease in chain length and loss of methyl groups probably occurs during the softening period and accounts for the rise in soluble pectin.

Pears are picked in the hard stage and held at low temperatures until required for ripening. On return to room temperature they rapidly ripen and soften. If pears are held too long at low temperatures, 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 the soluble pectin.

During cold storage inactivation of pectic enzymes slowly occurs and normal hydrolysis of protopectin does not take place. Due to this, in cold storage the amount of protopectin increases. Bananas undergo loss of protopectin and increase in the smaller molecules which make up soluble pectins during ripening. Loss of protopectin and rise of soluble pectins have been found in peaches, plums and tomatoes as they ripen. Changes in cellulose, hemicelluloses and lignins have been followed in a small number of fruits and with a few varieties.

13.4.5 Changes in Organic Acids:

Organic acids decrease in apple pulp and in pears during storage. Both of these fruits contain a large number of organic acids in low concentration, a small amount of citric acid and larger amounts of malic acid. The changes in organic acids during storage should be of considerable interest, since many organic acids are related to metabolic processes.

13.5 PLANT TISSUES:

A fresh fruit or vegetable at the time of harvest it is separated from tissues of the plant supplying water and other nutrients, it is still living. The cell in the edible portion of most fruits and vegetables is the parenchyma cell. It builds up the bulk of the cells in leaves, fruits and even in young edible stems. A parenchyma cell is rather thin walled. It may be polygonal or cubical in shape, but all are about the same size. The parenchyma cells do not fit tightly together but are often separated by air spaces that contribute to the slightly chalky appearance of a fresh fruit or vegetable.

The walls of parenchyma cells in young plants are composed almost entirely of fibrils of cellulose. The cells are held together by adhesive substances, which in the young plant are composed of pectic substances. As the plant grows older the nature of these adhesive substances often change, lignins and other compounds are deposited, and the cellulose layer of the cell wall thickens. Such an old plant is not considered desirable for food since it is woody and tough even when cooked.

The material within the cell wall is protoplasm, composed of a very large number of different molecules that form either a viscous fluid or a gel, such as proteins are colloidally dispersed. The protoplasm is not uniform but differentiated into various regions and cell

parts, the most distinct of which is the nucleus. The activity of a cell is directed by the nucleus and that a cell cannot survive long without it. Within the cytoplasm numerous small bodies called plastids are present. The parenchyma cells of leaves and occasionally other tissues contain granular green plastids called chloroplasts, the site of the chlorophyll so important in photosynthesis. If the plastid is any other colour because of the occurrence of other pigments in it, it is called chromoplast. The leucoplasts are colourless bodies containing starch granules.

Beside the plastids there are often large vacuoles in cells, made up of droplets of solutions with strands of cytoplasm around them. They contain salts, sugars and other soluble materials dissolved in water. This solution is sometimes called as the cell sap. In young cells the vacuoles are small and numerous. As the cell grows the total size of the vacuoles increased more rapidly than does the amount of protoplasm, through the imbibition of water and other small molecules. The vacuoles merge together and become much larger in size and smaller in number with often only one large vacuole in a mature cell. Cells with a high fat content may also have an oil vacuole. Some cells contain crystals embedded in the cytoplasm.

Other types of plant cells besides parenchyma cells are the conducting cells, the supporting cells, and the protective cells. The conducting cells are composed of long tubes through which water and salts or food stuffs are distributed through the plant. These are of two types called xylem and phloem. The walls of the xylem are composed primarily of cellulose thickened at intervals in definite patterns with lignin. The walls of the phloem contain little lignin. Fibers composed of cellulose may occur associated with the phloem. When numerous or large fibers are unchanged on cooking and produce stringiness and toughness.

The supporting tissues are not numerous in young plants or in the young parts of plants, desirable for foods. They are composed of long pointed cells whose cell walls of cellulose thicken as the plant ages and become coated with lignin. Some plants have another type of supporting cell in which the cell wall is composed of cellulose and pectic substances in place of cellulose and lignin.

The protective tissue is composed of specialized parenchyma cells that secrete cutin or contain suberin. Sometimes these cells are thick and corky, in other plants they are thin. But they are closely pressed together and are usually quite tough. Usually the epidermis of the fruit or leaf contains stomata, minute valves through which exchange of gases can occur when they are open. The cutin or suberin of the epidermal cells make them impermeable to water and also protect them from injury. This layer of cells forms a skin or peel which may be removed in the preparation of the leaf or fruit for eating. The layer of epidermal tissue not only protects the organ from mechanical injury, but also prevents injuries by insects, broken the keeping time of a fruit is relatively short, whether it has been harvested or not.

The cells of a harvested fruit or vegetable are still living. It is only when food is cooked that cells are killed. If the food is frozen or kept for a long time, death of cells usually occurs.

13.6 TEXTURE OF FRUITS AND VEGETABLES:

The texture of a fruit or vegetable depends on the turgor of the living cells as well as on the occurrence of supporting tissues and the cohesiveness of the cells. "Turgor is that pressure of the cell contents on the partially elastic wall of a cell, tending to produce rigidity". It is produced by a delicate balance of forces which maintains the cell at a normal volume however allows the exchange of substances.

When the cell volume diminishes, the cell becomes soft and flaccid, but if the volume increases beyond the point that can be accommodated by the elasticity of the cell walls, the cell ruptures, the cell contents flow out, and rigidity is lost. The substance chiefly responsible for changes in volume is water. When plant wilts water has been so extensively lost from the cells that they no longer have normal turgor but are soft and flabby.

One of the best known forces affecting cell volume is osmosis. Plant cell walls are composed of cellulose and permeable to many types and sizes of molecules. Inside the cell, protoplasm may be stretched around a large storage vacuole.

The protoplasm and cell wall act as a semipermeable membrane, allowing water and some other small molecules to pass through it. Water diffuses in greater amounts from a region in which it is high in concentration.

The vacuole contains soluble compounds as well as colloidal substances, and if the intercellular fluid is composed only of water or of a dilute solution, water will move into the cell. While the fruit or vegetable is still part of the plant, a fine balance is achieved and the volume of the cell is maintained at a certain normal turgor. The effect of a concentrated solution on cell turgor is readily demonstrated by placing cucumber slices in a concentrated salt solution or sprinkling sugar on strawberries. Both soon become flaccid as water rapidly passes from the cells in to the solution.

13.6.1 Factors Influence the Cell Turgor:

Cell Turgor is Influenced by Several Factors:

- The concentration of dissolved and colloidally dispersed substances within the vacuole,
- The permeability of the protoplasm, and
- The elasticity of the cell walls.
- Highly elastic cell walls allow for significant cell expansion before rupture, while strong, rigid walls maintain firmness even with cell volume shrink.

When a fruit or vegetable is cooked, the protein is denatured, the cells die, and the vacuoles are no longer covered by a living membrane of protoplasm. The protein usually precipitates and the permeability is significantly affected. Often solutes and water come out of the vacuoles into the intercellular spaces or even out of the fruit or vegetable and the food becomes very soft. However, when starch granules swell, become gelatinized and retain moisture. If there are sufficient of them and if the process continues, they may hold the water in the vacuole and maintain a firm texture. However cooked starch granules are never able to maintain crispness, this is noticeable in a sweet potato, peas and in beans.

The rigidity of the structural tissues and of the cell walls is an important factor in the texture of a fruit and vegetable.

13.7 SUMMARY:

Fruits and vegetables contain a variety of key biochemical components, including sugars, acids, fats, flavor compounds, phenols, vitamins, and minerals, all of which significantly contribute to their composition and quality. These components are essential for human nutrition. After harvest, fruits continue to undergo chemical changes, ultimately leading to spoilage caused by bacteria, fungi, or yeast. These chemical reactions directly impact the fruit's edibility and commercial value. Post-harvest changes in fruit are numerous, affecting respiration, water content, carbohydrates, organic acids, and pH.

13.8 TECHNICAL TERMS:

Postharvest changes, Respiration, Water loss, Texture.

13.9 SELF ASSESSMENT QUESTIONS:

1) Write about the chemistry of fruits and vegetables?
2) Explain detail about post harvesting changes in fruits and vegetables?
3) Discuss about the composition of fruits and vegetables?

13.10 REFERENCE BOOKS:

1) Food Chemistry by Lillian Hogland Meyer.
2) Postharvest Technology of Fruits and Vegetables (Handling, Processing, Fermentation and Waste Management) L.R.Verma and V.K.Joshi Volume 1, General Concepts and Principles.
3) A complete book on Fruits, Vegetables and Food Processing by Dr. H. Panda.

Dr. P. Kiranmayi

LESSON-14

PLANT PIGMENTS

14.0 OBJECTIVES:

After going through this lesson students will understand:

- Types of water insoluble pigments
- Chemical structure of water insoluble pigments and water soluble pigments
- Chemical changes on cooking

STRUCTURE:

14.1 INTRODUCTION:

The vibrant colors of fruits and vegetables are crucial to our enjoyment of food. Beyond their diverse and delicious flavors, it's the array of subtle and bright hues that makes them so appealing. These colors mainly come from pigments located within plastids, specialized structures inside plant cells. While pigments usually reside in plastids, they can sometimes appear as crystals within the cell protoplasm, like the carotene platelets in carrots

or the lycopene crystals in tomatoes. Water-soluble pigments, such as anthocyanins, are often dissolved in the cell vacuoles, giving a localized color rather than a dispersed one. Anthocyanins are particularly significant due to their widespread presence and their contribution of beautiful, bright colors to many flowers, vegetables, fruits, and grains.

Fruits and vegetables get their vibrant colors primarily from two main pigment groups:

Water Insoluble:

- Carotenoids
- Chlorophylls

Water Soluble:

- Anthoxanthins
- Anthocyanins

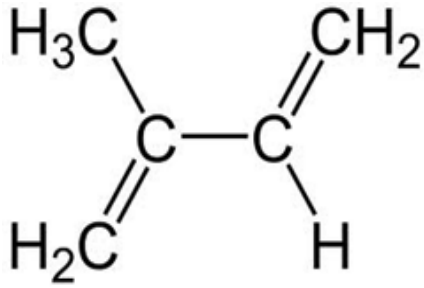
14.2 WATER INSOLUBLE PIGMENTS:

14.2.1 The Carotenoids:

Carotenoids are a group of fat-soluble pigments that create yellow, orange, and orange-red hues and are found abundantly in nature. In green leaves, they reside within the chloroplasts, small structures located near the cell walls of the palisade cells (those just below the leaf's upper epidermis). Carotenoids exist alongside chlorophyll within the lipid material of these chloroplasts. The dominant green of chlorophyll usually overshadows the carotenoids' yellow to red coloration, except in very young leaves where chlorophyll levels are low. The vibrant yellow-green of new spring leaves is due to the combined presence of carotenoids and small amounts of chlorophyll. These pigments are also prevalent in a wide range of fruits (like peaches, bananas, red peppers, paprika, tomatoes, and squash), other plant parts (carrots and sweet potatoes), and most yellow, orange, and red flowers. Animals that consume carotenoids tend to store them in their lipids, leading to their presence in blood, milk, egg yolks, and fat deposits.

14.2.2 Chemical Structure:

The name carotenoid is applied to all pigments chemically related to the carotenes, which were first isolated in 1831 by Wackenroder extracted from carrots and called the fraction carotene. Carotene is a mixture of three isomer, α, β and γ carotene. The carotenoids are either hydrocarbons or derivatives of hydrocarbons 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.

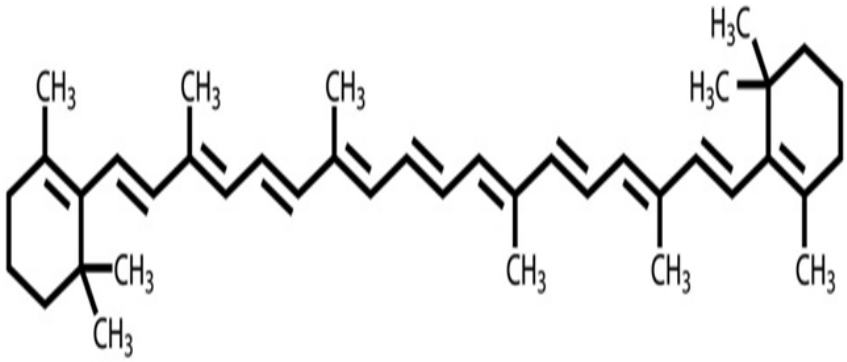


Isoprene

Some of the molecules contain a long unsaturated hydrocarbon chain with a ring at one or both ends of the chain. Some of the molecules are symmetrical, so that if folded in half, the left half would be the mirror image of the right half. β-carotene and lycopene are example of symmetrical molecules.

β-carotene and lycopene differ only in the cyclization of the end carbons of lycopene to form the rings of β-carotene. β-carotene is widely distributed in plant materials. It is readily prepared from carrots, sorb apples or paprika. It sometimes occurs free, although it is accompanied by small amount of α and γ-carotene. Lycopene is the orange red pigment of the tomato but it is also found in rosehips, water melon, apricot and many other plants. It also is usually accompanied by other carotenoids.

Some carotenoids have two terminal rings or groups which are different. Examples are α-carotene and γ-carotene. The difference between α-carotene and β-carotene is the presence of the double bond in ring 2. Unlike these pigments γ-carotene has only one ring and half of its molecule is like lycopene and half like β-carotene. In very few plant products α-carotene predominates. For example, in red palm oil it accounts for approximately 30 to 40 percent of the carotenes present.



β-carotene

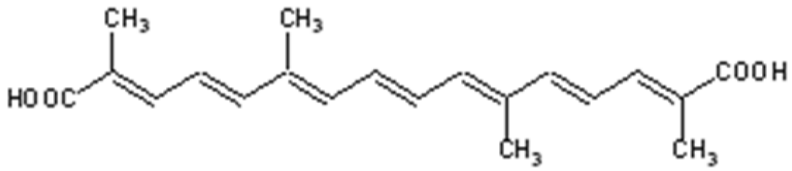
14.2.3 Xanthophylls:

"Carotenoids containing hydroxyl groups are called as xanthophylls". These often occur alongside carotenes. Leaves, for instance, contain both hydrocarbon carotenes (as their yellow pigments) and the related xanthophylls.

Cryptoxanthin, a common xanthophyll, shares the same isoprene-based structure and conjugated double bond system as other carotenoids. It's a major pigment in yellow corn, paprika, papaya, and mandarin oranges.

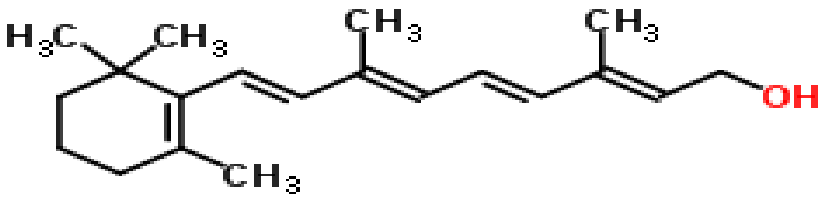
Crocetin:

Crocetin, a shorter-chain pigment found in saffron, exists as a glycoside. Saffron's distinctive deep orange-yellow color isn't solely due to crocetin; it's a blend of several carotenoids, including lycopene, β-carotene, γ-carotene, and zeaxanthin.



Crocetin

Certain carotenoids play a vital nutritional role as they can be converted into vitamin A within the body. Vitamin A1's structure is essentially half of a β-carotene molecule with an added hydroxyl group. Animals can efficiently convert each β-carotene molecule into two molecules of vitamin A through hydrolysis.



Vitamin A

14.2.4 Chemical changes during Cooking:

Carotenoids, being fat-soluble, are insoluble in water but readily dissolve in lipids and lipid solvents. This insolubility in water means minimal pigment loss during cooking or canning. However, they are susceptible to oxidation when exposed to air, posing a challenge during fruit and vegetable drying. Apricots and carrots, for example, can lose significant pigment during this process. While carotenoids don't typically undergo hydrolysis (except when present as esters in plant tissues), they are stable across a wide pH range.

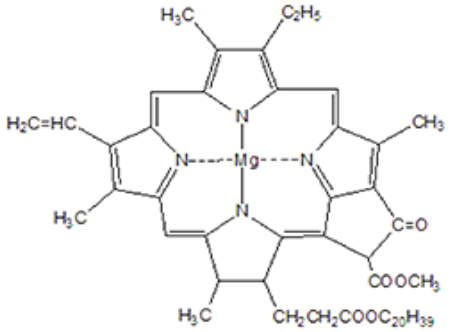
Blanching, especially followed by dehydration, can lead to rapid carotenoid loss in carrots. Diced carrots stored in humid air post-blanching can lose all their pigment within 20

hours. Generally, however, carotene losses during processing are relatively small (5-10%). Because of their vibrant color and relative stability, carotenoids are easily preserved during food processing.

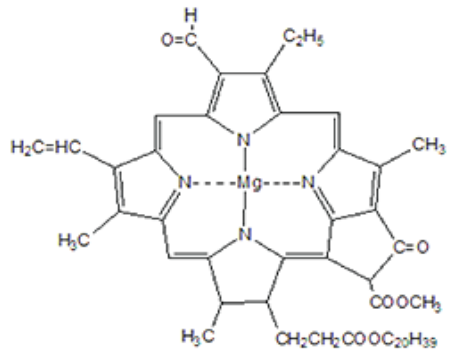
Carotenoids in carrots reside within chromoplasts. Some carrot cells also contain starch granules that may partially or fully enclose the carotene crystals. The highest concentration of carotene is found in the root's outer cells, where starch is minimal or absent, and the carotene appears as crystals (needles, tubes, flakes, or spirals) alongside tiny fat droplets. When these cells are killed (by blanching, drying, or chemical treatment), the chromoplasts break down, and the carotene dissolves into the oil droplets, which may already be present or may form after cell death.

14.2.5 Chlorophylls:

Chlorophylls are the green pigments of leaves and stems. Two types of chlorophylls have been isolated, chlorophyll a and chlorophyll b. Chemically, they are very similar. They belong to the group of biological pigments known as porphyrins, which includes haemoglobin. They are fairly large molecules composed of four pyrrole rings held together by, methane carbon (-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.



Chlorophyll a



Chlorophyll b

Chlorophyll

The chlorophylls are of great importance in the plant because of their role in photosynthesis and the formation of carbohydrates from carbon dioxide and water.

14.2.6 Changes during Cooking:

The chlorophylls are very unstable molecules when the living cell is killed, the chemical and physiochemical relations in a cell are changed. The chlorophylls are difficult to retain during any food processing and special care must be taken to produce food that retains a bright, attractive green colour. In living cells, the chlorophylls exist as protein complexes and when the cell is killed by heating, the protein is probably denatured and the chlorophyll released. This may account for the rapidity with which chlorophyll reacts in cooking food such as green beans.

During cooking, chlorophyll changes to an olive green colour and to brown. This is due to the replacement of hydrogen for the magnesium which has been complexed in the porphyrin to form pheophytin. When green beans are first dropped into boiling water, 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 a slight degree. Then as the bean is warmed, air is expelled and the intercellular spaces collapse or partially collapse. As cooking continues, the plant acids are liberated and the reason is chlorophyll is released from the protein complex or because the membrane around the chloroplasts becomes more permeable or both, the acid 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 escape during the early part of the cooking, colour retention is much better.

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 if the cation is sodium or potassium, cellulose hydrolyzes rapidly and the texture of the vegetable becomes very soft and mushy. Some of the vitamins like ascorbic acid and thiamine are very sensitive to heating at high pH’s. Addition of sodium bicarbonate in cooking water accelerated the rate of destruction of these vitamins.

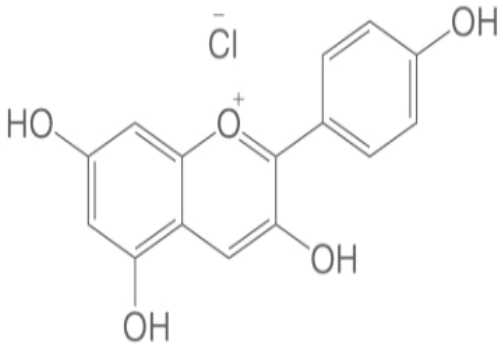
14.3 WATER SOLUBLE PIGMENTS:

14.3.1 Anthocyanins

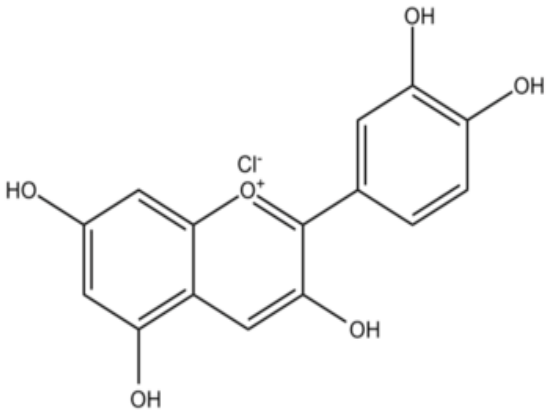
Most of the red, blue and violet pigments that occur in flowers, fruits, and other parts of plants belong to the group of pigments known as anthocyanins. These occur in plant cells as glycosides which are either of monosaccharides sometimes with one monosaccharide moiety

and sometimes withtwo. The colour results from the structure of the anthocyanidin which is combined with the monosaccharides. The carbohydrates commonly bonded to the anthocyanidins are glucose, rhamnose and occasionally a pentose. Most of the anthocyanins are soluble in water.

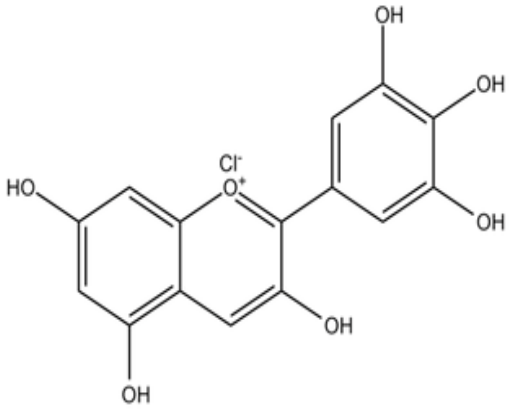
Only three types of anthocyanidins are identified in plant tissues. They are pelargonidin, cyanidin and delphinidin.



Pelargonidin chloride



Cyaniding chloride



Delphinidin chloride

The great variety of colours, hues and tints that occur in nature and the delicate shading on the cheek of a 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 pHs the anthocyanins pass through a violet and then blue colour. Some turn green and then yellow at very high pHs. Tannins are generally associated with anthocyanins and alter the colour. 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.

14.3.2 Changes during Cooking:

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 the 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 there is no loss of pigment. So frozen red raspberries show excellent retention of the pigment but in canned berries the colour gradually passes out in to the canning solution until the berries are practically colourless. When beets are cooked without removing the skins or even cutting off the root, colour retention is much better than when they are peeled and cubed. When grapes are hot pressed, most of the colour is removed in the juice and if they are fermented in wine making for a short time before the pressing, the extraction is almost complete.

The effect of changes of pH on the colour of the anthocyanins is frequently noticed in food preparation. Most fruits contain sufficient acid so that the pigment remains red or bluish red through the cookery or processing. But if a small amount of the juice is added to dish water containing soap or detergent and with a pH of 8 or 9, a blue or greenish blue colour forms. 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, blue colour formation is observed.

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 canning and in cookery. When tin cans are used for canning those fruits or vegetables containing anthocyanins, it is necessary to polish inside of the can. Tin cans without this polish will cause a discolouration of the fruit touching the side of the can.

Cooking fruits for jams and jellies in aluminium vessels, change in colour may be the result of the reaction with aluminium 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 a red-brown and finally a brown colour.

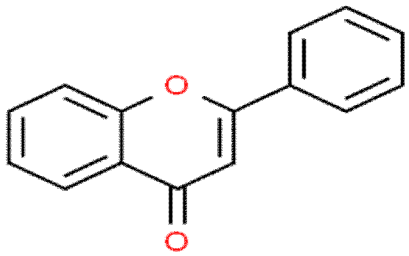
Storage temperature is most important in the rate at which the change in colour occurs. The deterioration in the colour of glasspacked pears, peaches, plums and grape juice is more dependent on high temperatures and oxygen content than light. Ascorbic acid protects the pears, plums and peaches against discoloration and the development of off flavours, but itdoes not protect grape juice.

14.3.3 The Anthoxanthins and Flavones:

One of the most important groups of pigments in plants are the anthoxnathins and the flavones. They are yellow pigments usually dissolved in the cell sap.

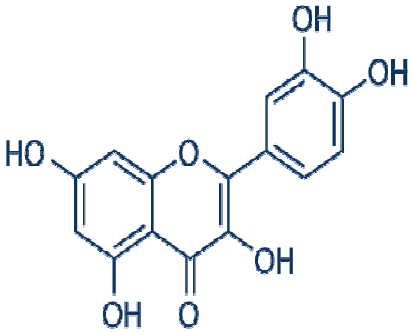
Chemical Structure:

The anthoxanthins are glycosides which on boiling with dilute acid yield one or two molecules of monosaccharides and a flavone or a derivative such as flavonal, flavanonal, or isoflavone.

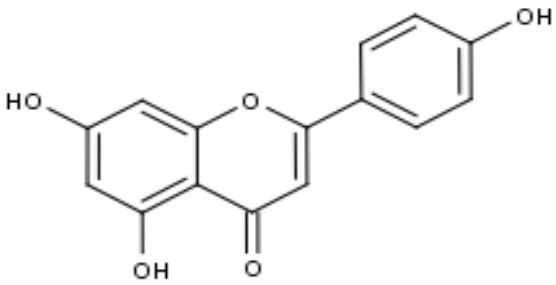


Basic Ring Structure of Flavone

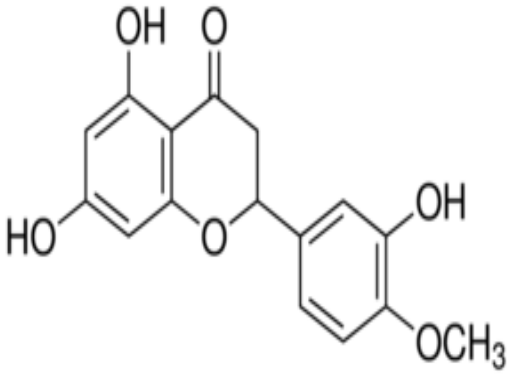
These pigments are dissolved in the cell sap and are usually pale yellow or colourless but occasionally bright orange. Most bright yellow or orange fruits and vegetables are coloured by carotenoids rather than anthoxanthins and flavones. The number of flavones and anthoxanthins isolated from plants is large (18 flavones, 27 flavonols, 5 flavononols and 14 isoflavones). Many of these are from plants or parts of plants not used as food. Examples of some of the flavones which occur in this group are



Quercetin



Apigenin



Hersperitin

Quercetin occurs in onion skins, tea, hops, horse chestnut, red rose, the bark of the American oak and many other tissues. Apigenin is present as glycoside in parsley and is also one of the pigments of the yellow dahlia. Hesperitin occurs in oranges and lemons as a 7-rhamnoside.

14.3.4 Changes in Cooking:

When a food is cooked in alkaline water, yellow or cream colour development is observed. Hard water will +have pH as high as 8 and soft water which contains $NaHCO_3$ in place of $Ca(HCO_3)_2$ will have a pH even higher. Potatoes cooked in soft water has 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, bands of yellow are sometimes observed where the pigment is more concentrated in some cells. Rice also shows this yellowing when cooked in soft 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 variety. The flesh turns pale yellow and the cooking water is bright yellow when alkaline water is used. Cauliflower and cabbage, sometimes show yellowing, occasionally cauliflower turns a brown or pinkish brown colour on cooking. Part of the colour may be caused by flavones, but most is the result of reactions of ions with the tannins.

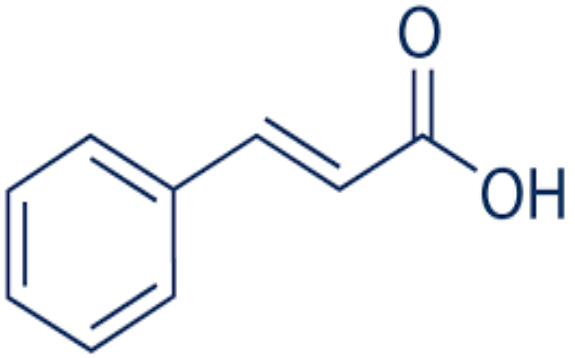
Tea contains both the flavone, quercetin and tannins. The change in colour has probably been noticed when the acid of lemon juice causes a fading of the colour.

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

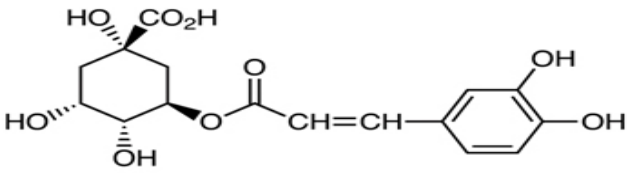
The flavonoids are a group of compounds widely distributed in the plant kingdom. They are water soluble and are often present in the juices of plants. Chemically the flavonoids contain two benzene rings with a three carbon bridge is condensed through an oxygen into an intermediate ring. The benzene rings hold hydroxyl groups.

The true flavonoids consist of the anthocyanins which are the red-blue purple pigments of plants. The anthoxanthins are yellow in colour. The catechins and the leucoan thocyanins are colourless but readily change to brownish pigments. They are probably called food tannins.

Compounds related to the flavonoids are numerous and are also widely distributed in nature. They are cinnamic acid, and chlorogenic acid.



Cinnamic acid

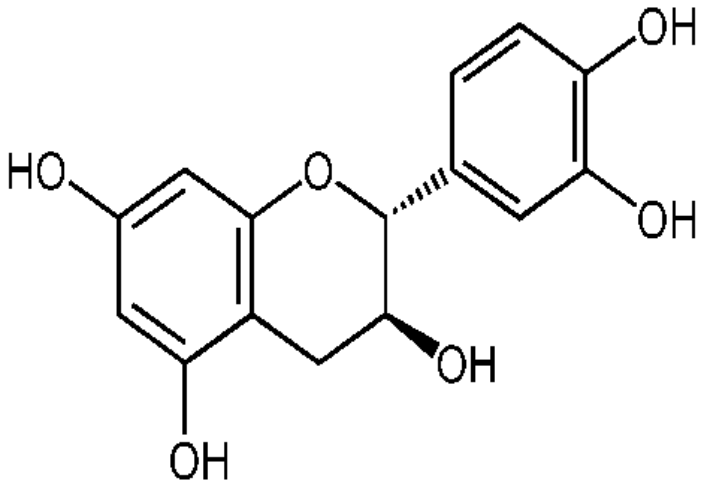


Cholorgenic acid

14.3.5 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. In ancient times tanning materials isolated from plants became objects of commerce. The tannins react with a number of ions and form dark colours which have been usedfor inks. They are readily oxidized with permanganate.

Dark colours and astringent tastes were attributed to tannins. The tannins of foods appear to be comprised of the catechins, the leucoanthocyanins, and some hydroxyl acids. All of them give colours with metal ions. Catachin and epicatechin are reduced derivatives of flavones. They are isomers in which the ring and hydroxyl groups are probably*trans* in catechin and *cis* in epicatechin. The structure of the leucoanthocyanins is still uncertain but they are perhaps closely related to thecatechins.



Catechin

Catechins and leucoanthocyanins are present in tissues of those woody plants such as apples, peaches, grapes, almonds, and some pears but they are absent in herbaceous plants. They are present in cereals although the amounts vary. Tea contains a number of compounds of catechin and epicatechin esterified with gallic acid.

14.3.6 Changes during Cooking:

If catechin is heated with dilute mineral acids, it forms an amorphous red precipitate which is highly insoluble. This is called "tannin red" or "phlobaphene" and is said to be polymer of the tannin.

When the tea and coffee are prepared 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. Instead of a clear, sparkling infusion, the tea or coffee is distinctly muddy. In iced tea this change is particularly observed, and with some water the beverage may be full of precipitate that it is opaque. The precipitate clings to the side of the cup or glass, and the

colour is visible. These transformations are believed to be caused by the reactions of the tannins in the tea and coffee with the calcium and magnesium ions of the water. If iron is present, very dark complexes are formed. Whether the precipitate is a simple calcium or magnesium salt of a tannin or it is a complex is not known. The change in colour which occurs when lemon juice is stirred in to black tea is also believed to be result of a change in the tannins.

A greenish gray discoloration occasionally occurs in chocolate ice cream and has been accredited to iron tannates formed from specks of rust from the ice cream cans and tannins in the cocoa.

When evaporated milk is allowed to stand in an opened can for some days, it imparts a grayish green colour to coffee. It depended on the formation of rust in the can. The colour is proportional to the amount of iron in milk. This is probably a reaction of the iron ions with the tannins of the coffee.

Dark spots on canned sweet potatoes are attributed to reaction of tannins with the iron ions formed from the walls of tin cans. A gray colour in sugar cane occur when iron ions from the crushers react with tannins.

The presence of tannins in foods sometimes gives body and fullness of flavour to the food. The greatest difference in composition between cider apples and culinary apples is in the tannin content. The cider produced from those varieties with relatively high tannin content has more astringency while that from culinary apples. The grapes must be removed from the stems before fermentation begins so that excessive astringency is avoided. Also the wine cannot be allowed to remain in contact with the seed for too long during fermentation, or too much tannin is extracted.

14.4 SUMMARY:

Most of the water insoluble pigments occur in plastids. Plastids are specialized bodies lying in the protoplasm of the cell. Occasionally a pigment is present in the protoplasm as a crystal. Those pigments include carotenoids, chlorophylls, anthoxanthins, and anthocyanins. The carotenoids are insoluble in water but soluble in lipids and in lipid solvents. In processing of fruits and vegetables, loss of these pigments into cookery or canning water is very slight. They do undergo oxidation when exposed to air. Chlorophylls are the green pigments of leaves and stems. Two types of chlorophylls have been isolated, chlorophyll a and chlorophyll b. During cooking, chlorophyll changes to an olive green colour and to brown. 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. 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 the water.

14.5 TECHNICAL TERMS:

β-carotene, Carotenoids, Xanthophylls, Chlorophylls, Anthocyanins, Anthoxanthines, Tannins, Flavones

14.6 SELF ASSESSMENT QUESTIONS:

1) Write about the types of water insoluble pigments and their chemical structure?
2) Discuss about the chemical changes occur in water insoluble pigments during cooking?

14.7 REFERENCE BOOKS:

1) Food Chemistry by Lillian Hogland Meyer.
2) Postharvest Physiology and Biochemistry of Fruits and Vegetables by Elhadi, M., Yahia, Armando Carrillo-Lopez.
3) Hand Book on Natural Pigments in Food and Beverages: Industrial Applications for Improving Food Color, Edited by Reinhold Carle and Ralf M.Schweiggert.

Dr. P. Kiranmayi

LESSON-15

FOOD ENZYMES

15.0 OBJECTIVES:

After going through this lesson students will understand:

- Types of enzymes in food
- Functions of enzymes
- Food quality related enzymes

STRUCTURE:

15.1 INTRODUCTION:

During the storage of fresh foods, numerous enzyme-catalyzed changes take place. Enzymatic activity plays a significant role in influencing various major food categories, including: a. Cereals, legumes**,** and grains b. Meat, poultry, and seafood c. Fruits and vegetables d. Milk and dairy products e. Chocolate, soft drinks, and wine f. Candy and confectionery.

In preprocessed foods, enzymes remain active and can impact product quality in various ways. Additionally, enzymes are widely utilized in food manufacturing, with processes such as cheese production and high-fructose syrup synthesis being notable examples.

Food enzymes are categorized into two main types:

- **Endogenous enzymes**: Naturally occurring in foods, influencing their quality and characteristics.
- **Exogenous enzymes**: Primarily derived from bacteria and intentionally added as processing aids in food production.

15.2 FUNCTIONS OF ENZYMES:

Enzymes play a crucial role in various biological processes within living organisms. They are vital for cell signaling and regulation, often functioning through kinases and phosphatases. Additionally, enzymes contribute to movement, as seen in myosin, which hydrolyzes adenosine triphosphate (ATP) to facilitate muscle contraction and transport cellular cargo along the cytoskeleton. The metabolic pathways within a cell are largely determined by the types and concentrations of enzymes present.

Enzymes are also essential for digestion in mammals and other animals. They help break down complex macromolecules into smaller, absorbable components. For example:

- Amylases hydrolyze starch into smaller sugars such as dextrins, maltose, and eventually glucose, which can be absorbed by the intestines.
- Proteases break down proteins into smaller peptides and amino acids.
- In ruminants, gut microorganisms produce cellulase, an enzyme that digests cellulose from plant fibers.

Enzymes often function sequentially in metabolic pathways, where the product of one enzyme serves as the substrate for another. In some cases, multiple enzymes can catalyze the same reaction, enabling greater regulatory control. Without enzymes, metabolic reactions would proceed at an insufficient pace to meet the cell's demands.

15.2.1 Enzymes are Biological Catalysts:

Enzymes act as biological catalysts, accelerating chemical reactions within living cells. They play a crucial role in enhancing reaction rates, facilitating hundreds of biochemical transformations essential for cellular function. In enzymatic reactions, a substrate is converted into a product through a specific catalytic process.

Enzymes are naturally occurring and can catalyze a wide range of organic reactions, including esterification, hydrolysis, hydrogenation, isomerization, oxidation, oxygenation, reduction, polymerization, and many others.

$$\textbf{Substrate(S)} \rightarrow \textbf{Product (P)}$$

$$\textbf{A+B} \rightarrow \textbf{C+D}$$

15.2.2 Enzymes are highly specific:

Enzymes exhibit high specificity, recognizing a single type of substrate or a group of closely related substrates, even in the presence of numerous other compounds. They function as highly efficient catalysts, operating effectively under mild conditions of temperature, pH, and substrate concentration. Their catalytic efficiency depends on strong substrate binding combined with rapid transformation on the enzyme's surface.

15.3 ROLE OF ENZYMES IN MAINTENANCE OF FOOD QUALITY:

Enzymes play a crucial role in modifying and enhancing the functional, nutritional, and sensory properties of food ingredients and products. Compared to chemical reactions, enzymes are highly specific, leading to fewer side reactions and byproducts, resulting in higher-quality products with reduced waste and pollution. Their ability to catalyze reactions under mild conditions helps preserve the valuable characteristics of food and its components.

Fresh food materials naturally contain a vast number of endogenous enzymes, which can cause both desirable and undesirable changes in food quality. When food becomes contaminated with microorganisms, biological spoilage occurs. Hydrolases are responsible for breaking down biopolymers such as proteins, polysaccharides, and lipids, leading to changes in the texture and consistency of raw foods.

15.3.1 Amylases:

Cereals are vegetable seeds and are rich in starch. The seeds use their starch to provide initial energy for germination and shooting / root formation to plants can start photosynthesis. Metabolism in the glycolytic system uses plants to generate ATP as an energy source. The common starting material for this energy production is glucose. Starch provides glucose through their hydrolysis in the germination stage. Amylases are enzymes that catalyze this hydrolysis and are activated and synthesized in the event of germination when water, oxygen, light and temperature conditions meet the requirements.

Enzymes that hydrolyze starch (glycosidic bonds)

- **α-amylase**
    a) Hydrolyses α-1-4 glycosidic bonds within starch
    b) Products are dextrins, maltose and maltotriose
- **β-amylase**
    a) Hydrolyses α-1-4 glycosidic bonds from the non-reducing end of starch
    b) Product is maltose (if amylose), what about with amylopectin?
- **Glucoamylase**
    a) Hydrolyses α-1-4 and α-1-6 (slower) glycosidic bonds in starch
    b) Possible to hydrolyze all the way to glucose

- **Pullulanase**
    a) Hydrolyses α-1-6 glycosidic bonds in starch (debranching enzyme)
    b) These enzymes are naturally present in the food or are found in microorganisms that are added to food

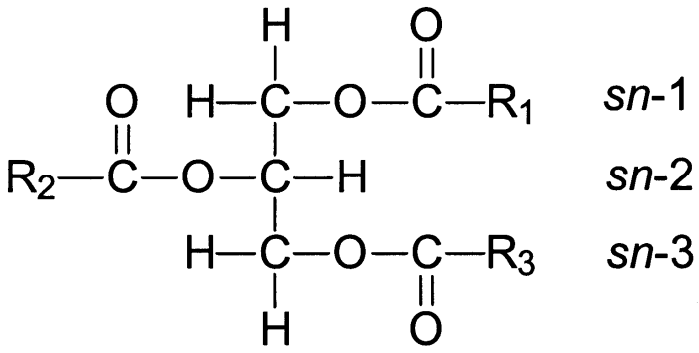
15.3.2 Proteinases:

These enzymes are often found in the transformation of traditional foods. Proteinases are used in the cheese manufacturing process. Cheese is a coagulation product of aged milk protein. Milk proteins are grouped into two groups: milk cell casein and protein. Casein proteins are in the form of colloidal particles (micelles) in milk, where α and hydrophobic β-casinines of the nucleus and hydrophilic casein κ are concentrated on the surface of the micelles. This structure allows α and β-casinines insoluble in milk. The thymosin hydrolytic proteinase κ-casein in Para hydrophobic casein destabilizes micelles in the aqueous phase. This leads to solidification of casein micelles with cheese formation.

Another traditional use of proteinases is found in tender meats. The meat is designed for 5 days to 2 weeks when the animal is packed. At this stage, the muscles mature with meat with native enzyme activity. In muscle, many different proteinases exist, with different specificities for different meat proteins and their working range. While aging continues, the metabolic activity remaining in meat produces lactic acid and pH gradually decreases. During this process, native proteinase works on various meat proteins, developing texture and taste changes in meat. This process can be enhanced by injecting exogenous enzymes into the meat during aging.

15.3.3 Lipases:

- Enzymes that hydrolyze bonds between the fatty acids and the glycerol molecule
- Hydrolyze triglycerides at the water-oil interface in emulsions
- Two classes
    a) 1,3-lipases: preferentially hydrolyze ester bonds at SN1 and SN3
    b) 2-lipases preferentially hydrolyze ester bonds at SN2



- Lipases have a dramatic impact on the quality of food products

A) Lead to Hydrolytic Rancidity

BAD when

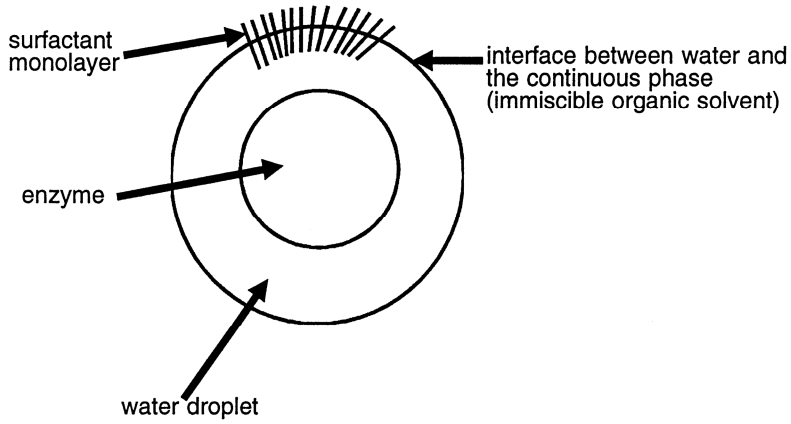
- Free fatty acids released in muscle foods and react to proteins to denature them and give a tough texture (happens on freezing muscle)
- They are not inactivated in milk; release short chain fatty acids that are very volatile and can also oxidize

GOOD when

- Used in fermented products
- Extremely important in ripening of cheeses and dry-sausages
- Short chain fatty acids released from milk fat produces the characteristic odor and flavor of these products (C:8 especially)

B) Lipases can be used to modify the properties of lipids

- Very popular application in the margarine industry to modify lipid crystal structure to give different textures and melting points
- Also used to produce mono and diglycerides for use as emulsifiers
- A very unique reaction system must be used for these enzymes since they are soluble in water but act on a lipid substrate

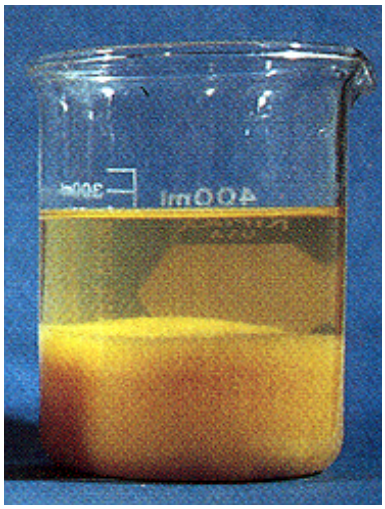


15.3.4 Pectinases:

Fruit juice is a product that is commonly found in food supplies and has been used for centuries. Home-produced juices are not opposed to medium yields and nubrens after many compress the fruit. However, the juice industry needs high yields from fruits and crystal-clear juice. The influential factor in these issues is the presence of pectin. Pectin is the polymers of sugarids. Pectin forms a skeletal fruit matrix. The matrix breaks the juice and resists pressure. Juice clouds are caused by released pectin substances. A juice press compresses the fruit and some of the itbone material is released from the fruit's flesh. Due to their hydrophobicity, the

released methoxes of galacturonan can be aggregated with other galacturonans and hydrophobic substances that form colloidal particles. These particles give the juice troubled appearance. To solve these problems, pectic enzymes have been used in the juice industry. The fruits generally give a small juice at a single press, but after treatment with pectic enzymes, 82% of the weight that juice can receive.

- Occur widely in fruits and vegetables and are responsible for the degradation of pectic substances
    - Pectin methyl esterase (PME)
        - Hydrolyze the methyl ester linkages of pectin
        - Causes loss of cloud in citrus juice (big problem)
        - Converts colloidal pectin to non-colloidal pectin
        - We add this enzyme when clarity is desired (e.g. apple juice), also has a minor effect on improving juice yield



15.3.5 Cellulases:

To extract juice, there is another substance that affects the meat of the fruits of the matrix known as cellulose. Hydrolysis of cellulose has been shown to increase fruit juice yield. When cellulose is used in combination with pectin enzymes, the enzyme mixture can incite the fruit completely. This process can be used to make spotless juices (a meaty residue left after the fruit has been crushed and the juice is extracted).

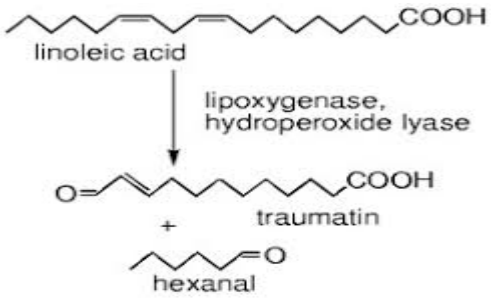
15.3.6 β-Galactosidases

Dairy products are one of the largest sectors in the food industry in many developed countries. The two main dairy products are food allergies and lactose intolerance. Lactose is the main carbohydrates found in milk. As soon as children are excommunicated from mother milk and start to eat other products, they reduce the production of the enzyme to lactose hydrolysis. If a person is not producing this enzyme sufficiently, the lactose (dairy product) swallowed will reach his large intestine.

Disaccharide assimilation is slower than monosaccharides, and therefore the remaining lactose is quickly absorbed by intestinal bacteria, causing carbon dioxide and lactic acid, and stimulates intestinal movements to cause diarrhea. β-galactosidase is an enzyme that hydrolyses the hydrolysis of galactose and glucose. By adding β-galactosidase to treat lactose milk, it can be hydrolyzed in milk. Resulted monosaccharides are easy to assimilate in the intestine and cause no diarrhea. This enzyme can also be used to produce sweetening agents from a byproduct of dairy processing.

15.3.7. Lipoxygenases:

- Found in a wide variety of plants (primarily legumes) and have also been identified in animal tissue (e.g. in the skin of fish)
- Specific for the oxidation of fatty acids that have a *cis, cis* penta-1,4-diene unit (methylene interrupted), so there are three naturally occurring fatty acids that can be substrates
    - Linoleic acid (2 double bonds)
    - Linolenic acid (3 double bonds)
    - Arachidonic acid (4 double bonds)
- Importance of lipoxygenases in foods
    - **Desirable**
        a) The enzyme plays a role in bleaching of wheat and soybean flours
        b) It contributes to the formation of S-S bonds in gluten in dough, thus one does not have to add chemical oxidizers to get stronger doughs
    - **Undesirable**
        a) Lipid oxidation and reactions of its products
        b) Breakdown products of hydroperoxides give off-flavors and odors
        c) Oxidation products (the free radicals or hydroperoxide) can bind and/or oxidize proteins to lead to textural problems
        d) Lipid oxidation also leads to nutritional loss of essential polyunsaturated fatty acids
        e) Vitamins may also be oxidized by the oxidation products
        f) Chlorophylls and carotenes (β-carotene) can be bleached
        g) Lipoxygenases can be effectively delayed by using antioxidants

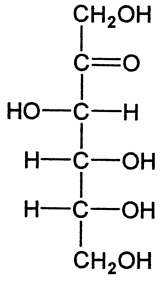


15.3.8 Glucose Oxidases:

Some oxidases are used in food processing to prevent oxidation. Oxidase glucose is used to remove in situ products, molecular oxygen, from hydrogen peroxide and low acids. Molecular oxygen is one of the most common oxidative agents living in processing and storage. Even when food is packaged in airtight containers to limit oxygen exposure, the small amount of oxygen already present inside the packaging and within the packaging materials themselves can still lead to food oxidation during storage. Removal of molecular oxygen in the package is a key factor in reaching long expiration dates. The glucose of oxidase can use glucose to capture oxygen. Small amounts of glucosidase can use glucose in food to complete the fatigue of molecular oxygen from dense airbags. Furthermore, since the products of this enzyme are gluconolactone and hydrogen peroxide, the products of in situ and hydrogen peroxide can be subjected to glucosexidase reactions. Oxidase glucose can be used to remove glucose to suppress Millard's reaction. This enzyme is also used to quantify glucose.

15.3.9 Glucose Isomerases:

- The most important isomerase for the food industry
- Catalyzes isomeric rearrangement of glucose to fructose (converts an aldose to a ketose)
- Gives a sweeter product than corn syrup
- Sweetness glu = 70; fru = 170; sucrose = 100
- Product called high fructose corn syrup (HFCS)
- Made from corn syrup (which is made by amylase digestion of starch)
- Enzymes are immobilized in large columns where the reaction takes place can reuse them



D-fructose

15.3.10 Rennet:

Rennet is considered to be a well-known exogenous enzyme used in the treatment of milk. Traditionally, animal rennet are used as coagulants in dairy industry milk for the production of high-quality cheeses with good texture and taste. Demand for cheese production is growing. At the same time, the supply of rennet calves decreased. This searched for alternative sources of rennet, such as rennet extracts from microorganisms. In the world, the rennet of microorganism constitutes 30% of the total produced cheese. Rennin has an enzymatic and not enzymatic action that coagulates milk. The milk transforms to a gel-like structure during the enzymatic activity due to the temperature and calcium ion effect.

15.3.11 Transglutaminase:

Transglutaminase (TGase) is the catalyst for the polymerization of dairy proteins and improves the properties of dairy products. Human lactose intolerance is due to inability to digest lactose due to a lack of secretion of lactase enzymes. Furthermore, transglutaminase sewing and calcium recovery have been studied to improve texture and bar storage with nutrient content rich in milk protein (MPC) and micellar casein concentrate (HPN). Concentrate (MCC). Hardness, baby, humidity, pH, color and activity were measured during accelerated storage.

Higher levels of Tgase cross linking improved HPN bar cohesiveness and decreased hardening during storage.

15.3.12 Xylanases:

Xylanases, along with α-amylase, glucososidase and protease, were used in malt amylase, preparation of bread, etc. This enzyme broke the hemicellulose of wheat flour by moving water, leaving milder dough, which will be easier to replace. He also set aside the formation of breadcrumbs and developed the dough. Chilanase is recommended to improve the texture of lighter cream crackers and give plate's uniformity and taste. Xylanases have also been used to reduce the viscosity of beer and dirty appearance by hydrolysis of arabinoxylose to reduce oligosaccharides. In combination with amylase, cellulus and pectinase, the Xylanase improves the yield by stabilizing the pulp of fruits, lightening fruits and vegetables, vitamins, mineral salts and edible colors. Other functions include increased recovery of essential oils, hydrolysis of substrate, reduction of viscosity etc. They delayed the physical and chemical juice clearing. Xylanases in combination with endoglucanase was used to separate starch gluten from wheat flour.

15.4 SUMMARY:

Enzymes serve as a wide variety of functions within living organisms. Enzymes such as amylase, large starch molecules break up; Proteases break large protein molecules. The result of these destruction reactions is the formation of smaller fragments that can easily absorb animal intestines. Steel enzymes can work together in a certain order, creating

metabolic tracks. Enzymes can modify and improve the functional, nutritional and sensory properties of ingredients and products. Enzymes can catalyze reactions under extremely soft conditions that do not destroy the valuable attributes of food and food components.

15.5 TECHNICAL TERMS:

Amylases, Rennet, Glucose oxidases, Xylanases, Cellulases, Pectinases.

15.6 SELF ASSESSMENT QUESTIONS:

1) What are the types of enzymes present in food?
2) Discuss about the functions of enzymes?
3) How enzymes maintain the quality of food?

15.7 REFERENCE BOOKS:

1) Richard owusu-apenten, introduction to food chemistry.
2) Robert J Whitehurst and marten vanoort, emzymes in food technology.
3) Principles of food chemistry third edition john M deMan, Ph.D.
4) Food Chemistry Encyclopedia Peter Varevlis, Lawrence Melton, Fereidun Shahidi.
5) Enzymes in food biotechnology: production, applications and future prospects, editor Mohammed Kuddus.

Dr. P. Kiranmayi

LESSON-16

DETERMINATION OF TOTAL ASH, VITAMINS AND MINERALS

16.0 OBJECTIVES:

After going through this lesson students will understand:

- Determination of ash content in foods
- Determination of vitamins and minerals

STRUCTURE:

16.1 INTRODUCTION:

Ash refers to the inorganic substances remaining after either ignition or complete oxidation of organic matter in a foodstuff. Dry ashing is mainly used for proximate composition and for some types of specific mineral analyses. Wet ashing (oxidation) is a preparation for the analysis of certain minerals. Now microwave systems are available for both dry and wet ashing, to speed up the processes. Most of the dry samples like wholegrain, cereals, and dried vegetables need no preparation, whereas fresh vegetables and high fat products such as meats need to be dried before subjected to ashing process. The ash content of foods can be expressed on either a wet weight or on a dry weight basis.

16.2 DETERMINATION OF TOTAL ASH CONTENT:

The inorganic material remaining after ignition of food material is termed as ash. Ashing is mainly of two types

a. Dry Ashing:

Dry ashing utilizes a muffle furnace, which can reach temperatures between 500 and 600 degrees Celsius. This process involves vaporizing water and other volatile compounds, while the organic components combust in the presence of air oxygen, transforming into carbon dioxide and nitrogen oxides. Elements such as Fe, Se, Pb, and Hg may partially volatilize with this procedure.

b. Wet Ashing:

It is a method for oxidizing organic substances by using acids and oxidizing agents. Minerals are solubilized without volatilization.

16.2.1 Dry Ashing:

Dry ashing is burning at high temperature (525°C or higher). Burning is carried out by a muffle furnace. A number of 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.

Procedure:

To determine ash content, begin by accurately weighing 5 to 10 grams of sample into a pre-weighed (tared) crucible. If the sample is particularly moist, dry it first. Carefully place the crucibles in a cool muffle furnace (or a warm one, using appropriate safety equipment like tongs, gloves, and eye protection). Incinerate the samples at approximately 550°C for 12 to 18 hours, or overnight. Once the incineration is completed, turn off the muffle furnace and

allow the temperature to drop to at least 250°C, ideally lower, before opening it. Open the furnace door slowly and carefully to prevent any light, fluffy ash from being lost. Using tongs, promptly transfer the crucibles to a desiccator containing a porcelain plate and desiccant. Cover the crucibles, seal the desiccator, and allow them to cool to room temperature before weighing. Warm crucibles can heat the air inside the desiccator, and with very hot samples, covers can be displaced allowing air to escape, or a vacuum can form upon cooling. Gradual pressure equalization is important. At the end of the cooling period, carefully and slowly slide the desiccator cover to one side to prevent a sudden rush of air. Desiccator covers with ground glass sleeves or fittings for rubber stoppers can be used for a controlled release of any vacuum. Finally, calculate the ash content by using below formula.

$$\% \text{ ash (dry basis)} = \frac{\text{Weight after ashing-tare weight of crucible}}{\text{Original sample weight X dry matter coefficient}}$$

Dry matter coefficient = % solids/100 for example, if corn meal is 87% dry matter, the dry matter coefficient would be 0.87. If ash is calculated on an as received or wet weight basis (includes moisture), delete the dry matter coefficient from the denominator. If moisture was determined in the same crucible prior to ashing, the denominator becomes (dry sample wt - tared crucible wt).

16.2.2 Wet Ashing:

Wet ashing, also known as wet oxidation or wet digestion, is primarily employed to prepare samples for the analysis of specific minerals and metallic poisons. Analytical labs often rely exclusively on wet ashing for certain mineral analyses (like iron, copper, zinc, and phosphorus) because dry ashing can lead to volatile losses of these elements.

Procedure:

Method-1:

In wet ash procedure concentrated nitric acid and sulfuric acid are used. In the first step, weigh a dried, ground 1g sample in a 125 ml Erlenmeyer flask. Prepare a blank of 3 ml of H_2SO_4 and 5 ml ofHNO_3, to be treated like the samples. (Blank is to be run with every set of samples.). Add 3 ml of H_2SO_4 followed by 5 ml of HNO_3 to the sample in the flask. Heat the sample on a hot plate at approximately200°C (boiling). Brown-yellow fumes will be observed. Once the brown-yellow fumes cease and white fumes from decomposing H_2SO_4 are observed, the sample will become darker. Remove the flask from the hot plate. Do not allow the flask to cool to room temperature. Slowly add 3 to 5 ml of HNO_3. Put the flask back on the hot plate and allow the HNO_3 to boil off. Proceed to the next step when all the

HNO_3 is removed and the colour is clear to straw yellow. If the solution is still dark in color, add another 3 to 5 ml of HNO_3 and boil. Repeat the process until the solution is clear tostraw yellow. While on the hot plate, reduce the volume appropriately to allow for ease of final transfer. Allow the sample to cool to room temperature, and then quantitatively transfer the sample to volumetric flask. Dilute the sample to volume with distilled water, and mix well. Dilute further, as appropriate, for the specific type of mineral being analyzed.

Method-2:

Evaporate moist samples (25 to 50 ml) in an appropriate dish at 100°C overnight or in a microwave drying oven until dry. Heat on a hot plate until smoking ceases. Ash the sample in a 525°Cfurnace for3 to 8 h. Remove dish from furnace and allow to cool. Ash should be grayish white to white and free from carbon. Cool and wet with deionized distilled water plus 0.5 to 3.0ml of HNO_3.Dry on a hot plate or steam bath and then return to a 525°C furnace for 1 to 2 h. Dissolve the ash in 5 ml of 1MHNO_3 by warming on a hot plate for 2 to 3 min to aid solution. Transfer to an appropriate size volumetric flask (i.e., 50 ml), then repeat with two additional portions of 1MHNO_3.

16.2.3 Microwave Ashing:

Microwave instrumentation offers a faster alternative to traditional ashing methods. Instead of conventional dry ashing in a muffle furnace and wet ashing using flasks or beakers on a hot plate, both processes can be performed using microwave technology. While conventional ashing can take several hours, microwave-assisted ashing can reduce sample preparation time to mere minutes.

Microwave Wet Ashing:

Microwave wet ashing or acid digestion, can be conducted safely in either open or closed vessel microwave systems. The system choice depends on the sample size and the necessary digestion temperatures. Closed vessels, capable of withstanding higher pressures (up to 1500 psi in some cases), allow acids to be heated above their boiling points. This elevated temperature and pressure lead to more complete dissolution of substances that are typically difficult to digest.

Closed vessel microwave digestion system:

In closed vessels specifically designed for high temperatures/ high pressure reactions, nitric acid can reach a temperature of 240°C.

Closed Vessel Microwave Digestion System

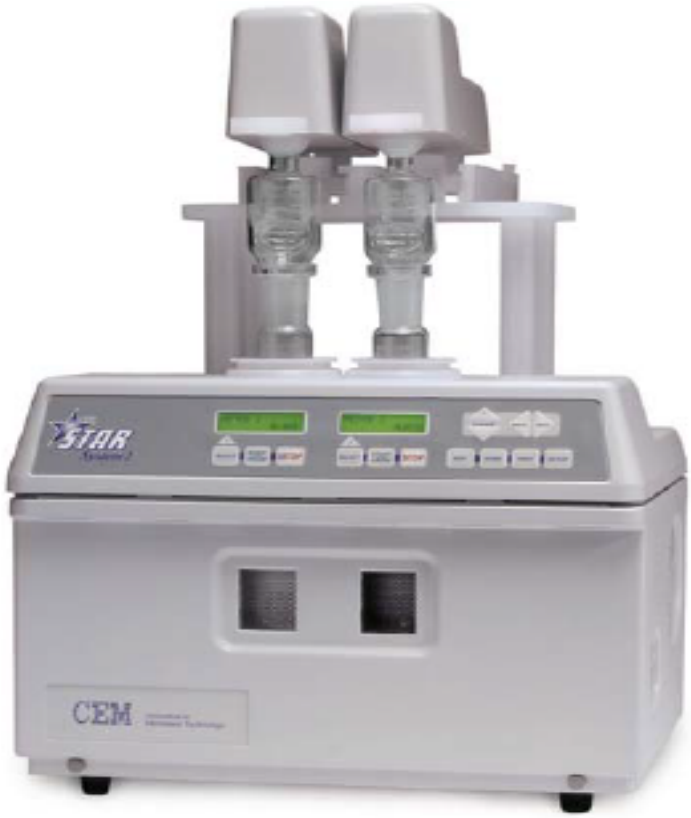
(Courtesy of CEM Corporation, Matthews, NC)

Thus, nitric acid is the acid of choice, though hydrochloric, hydrofluoric, and sulfuric acids also are used, depending on the sample and the subsequent analysis being performed.

The carousel is then 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 30 min. Because of the pressure generated by raising the temperature of a reaction, the vessels must be allowed to cool before being opened.

Open Vessel Microwave Digestion Systems:

These systems are used often for larger sample sizes (up to 10 g) and for samples that generate significant 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. Acid is automatically added according to the programmed parameters. Sulfuric and nitric acids are used frequently with open vessel systems, as they process reactions under atmospheric conditions. However, hydrochloric and hydrofluoric acids, as well as hydrogen peroxide, can be used. These instruments do not require the use of a fume hood, because a vapour containment system neutralizes harmful fumes.



Open Vessel Microwave Digestion System

(Courtesy of CEM Corporation, Matthews, NC)

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 vapour containment system neutralizes the fumes from the reaction. Samples are processed much faster and more reproducibly than on a conventional hot plate.

Microwave Dry Ashing:

Compared to conventional dry ashing in a muffle furnace that takes many hours, microwave muffle furnaces can ash the samples in minutes, decreasing analysis time by as much as 97%. Microwave muffle furnaces can reach temperatures of up to 1200°C. 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 (25 ml) crucibles ata time.

MICROWAVE MUFFLE FURNACE

(Courtesy of CEM Corporation, Matthews, NC)

In microwave dry ashing, a desiccated crucible is weighed and then sample is added and it is weighed again. Then the crucible is placed in the microwave furnace. The time and temperature parameters are set. The system is started and the program is run to completion. The crucible is carefully removed with tongs and reweighed. The sample may be further analyzed, if required. Some tests call for acid to be added to a dry ashed sample, which is then digested for further analysis.

16.3 DETERMINATION OF VITAMINS:

Vitamins are defined as relatively low molecular weight compounds. Any living organism that depends on organic matter as a source of nutrients, require small quantities for normal metabolism. With few exceptions, humans cannot synthesize most of the vitamins and therefore need to obtain them from food and supplements. Vitamins consist of a mixed group of chemical compounds and are not related to each other as are proteins, carbohydrates and fats. Their classification together depends not on chemical characteristics but on function.

Vitamins are different from the trace elements, also present in the diet in small quantities, by their organic nature. Vitamins are required in trace amounts (µg to mg per day) in diet for health, growth, and reproduction. Omission of a single vitamin from the diet of a species that requires will produce deficiency signs and symptoms. Many of the vitamins function as coenzymes.

Vitamins have been divided in to two groups based on their solubility in fat solvents or in water. Thus, fat soluble vitamins include A, D, E, and K, while vitamins of the B-complex and C are classified water soluble. Fat soluble vitamins are found in food stuffs in association with lipids. The fat soluble vitamins are absorbed along with dietary fats, apparently by mechanisms similar to those involved in fat absorption.

Water soluble vitamins are not associated with fats, and alterations in fat absorption do not affect their absorption. Three of the four fat soluble vitamins (vitamins A, D, and E) are well stored in appreciable amounts in the animal body. Except for vitamin B12, water soluble vitamins are not well stored, and excess are rapidly excreted. A continual dietary supply of the water soluble vitamins and vitamin K is needed to avoid deficiencies. Fat soluble vitamins are excreted primarily in the feces via the bile, whereas water soluble vitamins are excreted mainly in the urine. Water soluble vitamins are relatively nontoxic, but excess of fat soluble vitamins A and D can cause serious problems. Fat soluble vitamins consist only of carbon, hydrogen, and oxygen, whereas some of the water soluble vitamins also contain nitrogen, sulfur, or cobalt.

Analyzing the vitamin content of food and biological samples is essential for establishing nutritional requirements for both animals and humans. Accurate food composition data is also crucial for assessing dietary adequacy, understanding dietary intake, and ultimately improving global human nutrition.

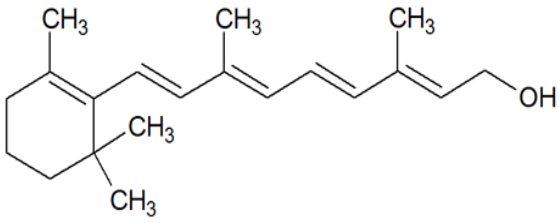
16.3.1 Bioassay Methods:

At present, bioassays are used only for the analysis of vitamins B12 and D. It is the reference standard method of analysis of food materials known as the line test, based on bone calcification. Because the determination of vitamin D involves deficiency studies besides sacrificing the test organisms. It is limited to animals rather than humans as test organisms.

16.3.2 Vitamin A:

Vitamin A is arguably the most crucial dietary supplement for all animals, including ruminants. While plants don't contain vitamin A directly, they do provide its precursors, carotenoids. Animals possess an enzyme in their intestinal walls that can convert these carotenoids into actual vitamin A.

Because, vitamin A is easily degraded by UV light, air, heat, and moisture, careful handling is essential. Protecting vitamin A involves using specialized low-actinic glassware, minimizing exposure to oxygen by using a nitrogen atmosphere or vacuum, avoiding high temperatures, and adding an antioxidant at the start of any procedure.



Vitamin A

https://commons.wikimedia.org/wiki/File:Vitamine_A.png

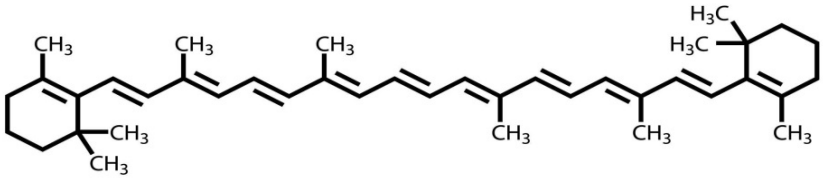
Determination of Vitamin A:

Biological methods include growth responses of rats or chicks, the storage test (liver). Physicochemical methods include colour reactions with antimony trichloride (Carr-Price method), gas chromatography, thin layer chromatography and spectrophotometric procedures.

High performance liquid chromatographic (HPLC) methods are the only acceptable methods to provide accurate food measurements of vitamin A activity. All work must be performed in subdued artificial light. Care must be taken to avoid oxidation of the retinol throughout the entire procedure. Solvent evaporation should be completed under nitrogen, and hexadecane is added to prevent destruction during and after solvent evaporation.

16.3.3 β-Carotene:

β-carotene is generally regarded as the most commercially important and widely used carotenoid. β-carotene is currently incorporated in a wide variety of dietary supplements, including multivitamin, vitamin A, and antioxidant formulations. Carotenoids have been shown to have biological actions independent of vitamin A. Some animal studies indicate that certain carotenoids with antioxidant capacities, but without vitamin A activity, can enhance many aspects of immune functions, can act directly as antimutagens and anticarcinogens, can protect against radiation damage, and can block the damaging effects of photosensitizers. β - carotene can function as a chain breaking antioxidant. It deactivates reactive chemical species such as singlet oxygen, triplet photochemical sensitizers, and free radicals, which would otherwise induce potentially harmful processes (eg. Lipid peroxidation).



β-carotene

https://www.chemistryworld.com/podcasts/beta-carotene/3005712.article

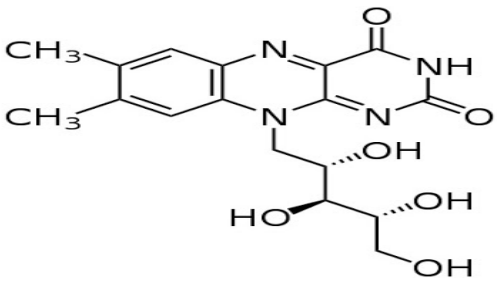
Determination of β-carotene:

Spectrophotometry is a common technique for the analysis of β-carotene. But spectrophotometric methods cannot differentiate between all-trans- β-carotene and cis-isomers of β-carotene, which may be formed during processing. Spectrophotometric analytical procedures are also not capable of determining β-carotene in combination products containing other carotenoids such as α-carotene, lutein, or lycopene. β-carotene and carotenoids in general have been intensively studied by liquid chromatography(LC), and procedures have been reported for the separation of β -carotene cis-/trans-isomers and for β-carotene in supplements and foods.

16.3.4 Riboflavin (VitaminB2):

There are three principle forms of endogenous vitamin B2 in foods: riboflavin (RF) and its physiologically active coenzyme forms, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Riboflavin is essential for energy production. Some of the physiological functions of the flavin coenzymes include: dehydrogenation, hydroxylation, oxidative decarboxylation, deoxygenation, oxygen reduction to hydrogen peroxide and electron transfer.

Riboflavin deficiency generally occurs in conjunction with other nutritional deficiencies because this vitamin is essential for the conversions of pyridoxine (vitamin B6) and folic acid into their active coenzyme forms, and for the conversion of dietary tryptophan into niacin.



Riboflavin

http://quimicaalkano.com/eng/product/vitamin-b2-riboflavin/

Determination of Riboflavin:

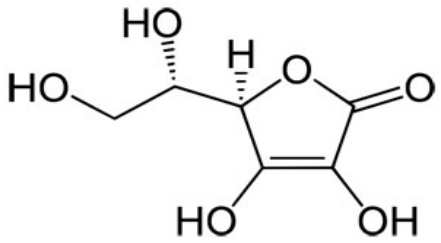
Due to the extreme sensitivity of the vitamin to UV radiation, all operations need to be conducted under subdued light. The analyst also needs to be aware that exact adherence to the permanganate oxidation process is essential for reliable results. In spite of the fact that

riboflavin is classified as a water soluble vitamin, it does not readily dissolve in water. When preparing the standard solution, the analyst must pay special attention and ensure that the riboflavin is completely dissolved.

For analysis, a shorter hydrolysis with cold trichloroacetic acid and no enzymatic hydrolysis has been used. For methods that lack a separation step, endogenous fluorescent substances in the food samples are oxidized to nonfluorescing compounds using potassium permanganate/hydrogen peroxide. Sodium metabisulfite converts riboflavin into its nonfluorescent leuco form and permits measurement of the background fluorescence. Solid phase extraction many also be used for sample purification prior to vitamin B2 quantification.

16.3.5 Determination of Vitamin C:

Vitamin C is widely known for its role in preventing scurvy. Vitamin C acts as a cofactor in hydroxylation reactions involved in collagen, norepinephrine and carnitine synthesis, and in steroid metabolism. Fruits and vegetables are the most common food sources of vitamin C. vitamin C exists naturally as two biologically active vitamers: L-ascorbic acid (AA) and dehydroascorbic acid (DHAA). The C vitamers, AA and DHAA are relatively small molecules. Both are readily soluble in water, but less soluble in organic solvents such as ethanol and acetonitrile and insoluble in less polar organic solvents. The most important chemical property of vitamin C is lack of stability.



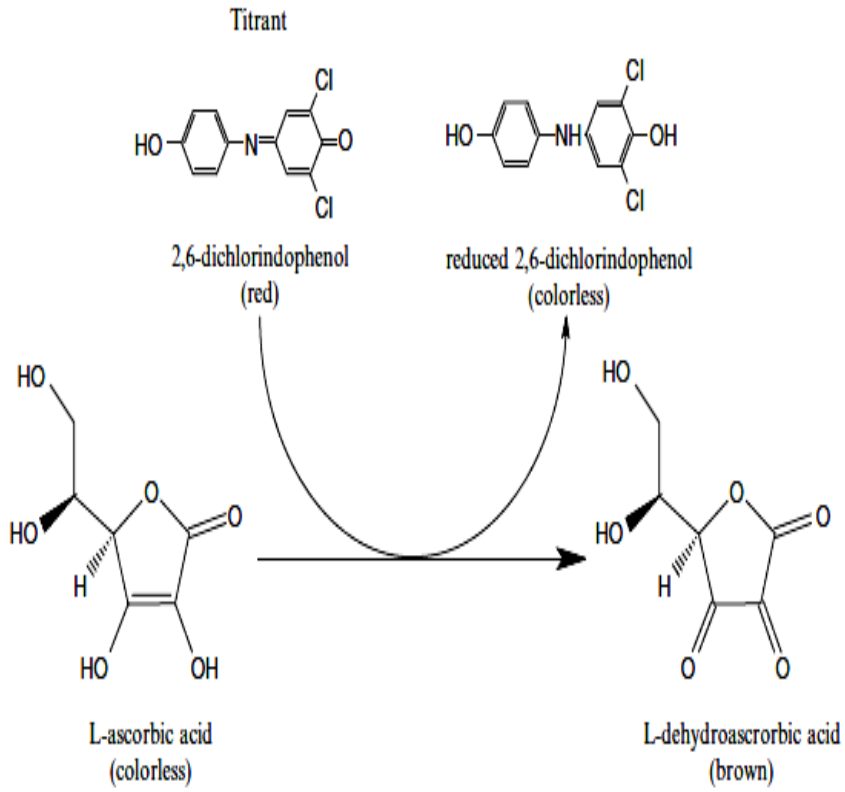
Vitamin C

https://www.worldofmolecules.com/antioxidants/vitaminc.htm

16.3.5.1) 2, 6-Dichloroindophenol Titrimetric Method:

L-ascorbic acid is oxidized to L-dehydro ascorbic acid by the oxidation-reduction indicator dye, 2,6-dichloroindophenol. At the end point, excess unreduced dye appears rosepinkin acid solution. In the presence of significant amounts of ferrous Fe, cuprous Cu, and stannous Sn ions in the biological matrix to be analyzed, it is advisable to include a chelating agent such as ethylene diamine tetra acetic acid (EDTA) with the extraction to avoid overestimation of the ascorbic acid content. The light but distinct rose pink endpoint should last more than 5sec to be valid. With coloured samples such as red beets or heavily browned products, the endpoint is difficult to detect by human eyes. In such cases it needs to

be determined by observing the change of transmittance using a spectrophotometer with the wavelength set at 545 nm.



Chemical reaction between L-ascorbic acid and the indicator dye 2,6-dichloroindophenol

16.4 ESTIMATION OF MINERALS:

Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the normal life processes. Plants, animals and humans need minerals in order to be healthy. Plants absorb minerals from the soil, and animals get their minerals from the plants or other animals.

The macro minerals include calcium, phosphorus, sodium and chloride, while the micro elements include iron, copper, cobalt, potassium, magnesium, iodine, zinc, manganese, molybdenum, fluorine, chromium, selenium and sulfur.

16.4.1 Determination of Calcium:

16.4.1.1) EDTA Complexometric Titration:

Principle:

Ethylene diamine tetra acetate (EDTA) forms stable 1:1 complexes with numerousmineral ions. This gives complexometric titration using EDTA in mineral analysis.

Stabilityof mineral-EDTA complexes generally increases with valence of the ion, although there is significant variation among ions of similar valence due to their coordination chemistry. The complexation equilibriumis strongly pH dependent. With decreasing pH the chelating sites of EDTA become protonated, there by decreasing its effective concentration. Endpoints are detected using mineral chelators that have coordination constants lower than EDTA (i.e., less affinity for mineral ions) and that produce different colours in each of their complexed and free states. Calmagite and Eriochrome Black T (EBT) are such indicators that change from blue to pink when they complex with calcium. The endpoint of a complexometric EDTA titration using either Calmagite or EBT as the indicator is detected as the colour changes from pink to blue. The pH affects a complexometric EDTA titration in several ways and must be controlled for best performance. The pH must be 10 or more for calcium to form stable complexes with EDTA. Also, the sharpness of the endpoint increases with increasing pH. Considering all factors, EDTA complexometric titration of calcium is specified at pH10 ± 0.1 using an ammonia buffer.

Procedure:

The calcium-calmagite complex is not stable, and calcium alone cannot be titrated using the calmagite indicator. However, calmagite becomes an effective indicator for small amount of neutral magnesium salt and enough EDTA to bind all magnesium. Upon mixing sample into the buffer solution, calcium in the sample replaces the magnesium bound to EDTA. The free magnesium binds to calmagite, and the pink magnesium-calmagite complex persists until all calcium in the sample has been titrated with EDTA. The first excess of EDTA removes magnesium from calmagite and produces a blue endpoint.

16.4.2 Determination of Phosphorous:

The method makes use of the fact that phosphorous can form a reducible complex with molybdic acid. The reduced complex, molybdenum blue, has a λ_{max} value of 650nm, at which the unknown sample can be measured. The reagents used are:

- Standard phosphate solution: Dissolve 0.4389 g of KH_2PO_4 in water and add 10 ml of 10N H_2SO_4. Dilute the solution to 1 litre in a flask. This solution contains 0.1 mg/ml of phosphorous.
- Molybdenum solution: Dissolve 25 g of ammonium molybdate reagent in 400 ml water. Add 500 ml of 10N H_2SO_4. Dilute to 1 litre in a standard flask with water.
- Reducing solution: Weigh 0.5g of aminonaphthol sulphonic acid, 30g of $NaHSO_3$,6g of Na_2SO_3. Dissolve in water, dilute to 250 ml and filter.

For colour development, take 5ml of ash solution in a 50 ml flask. Add 5 ml of molybdate reagent. Mix well and add 2 ml of reducing agent solution. Shake well and dilute to 50 ml with water. Keep for 10 minutes and measure the optical density at 650nm *vs* reagent blank.

For calibration curve, dilute 10ml of standard phosphate solution to 50 ml with water. Take between 5 to 40 ml aliquots of the solution, develop and measure the colour as above. Plot a calibration curve of OD *vs* concentration and find the unknown amount.

16.4.3 Determination of Iron:

Ferric ion forms a coloured complex with thiocyanate, ferric thiocyanate. The complex has λ_{max} value of 480nm. Any iron present in the sample as Fe (II) is oxidized to Fe(III) state with oxidants like H_2O_2 or $K_2S_2O_8$ solution. The reagents used are:

- Preparation of standard iron solution: Weigh accurately 0.702g of Fe(III) ammonium sulphate and dissolve in 100ml water to which 5 ml of concentrated H_2SO_4 is added. Dilute solution to 1 litre. This solution contains 0.1mg/ml of iron.
- KCNS solution
- Saturated $K_2S_2O_8$ solution

For colour development: Take 5 ml of sample ash solution in a 15ml flask. Add 0.5ml concentrated H_2SO_4, 1ml $K_2S_2O_8$ reagent and 2ml KCNS solution. Dilute to the required volume with water. Measure the OD at 480nm.

For standard curve: Take an aliquot of standard Fe(III) solution between 0.5 to 2.5ml. Develop and measure the colour as above. Plot a calibration curve and find the unknown concentration.

16.5 SUMMARY:

Traditional dry ashing involves high-temperature incineration in a muffle furnace, producing a residue that can be used for some mineral analyses (though not all). Wet ashing (or oxidation) is frequently used to prepare samples for specific elemental analysis, as it dissolves minerals while simultaneously breaking down all organic matter. Since humans can't produce most vitamins, they must obtain them through diet or supplements. Vitamin analysis of food and biological samples is crucial for determining nutritional needs in both animals and humans. For accurate measurement of vitamin A activity in food, HPLC is the preferred method. However, spectrophotometry remains a common technique for analyzing β-carotene in commercial products.

16.6 TECHNICAL TERMS:

Dry ashing, Wet ashing, Microwave ashing, EDTA Complexometric Titration, Vitamin A, β-Carotene, Ascorbic acid, Microfluorometric method

16.7 SELFASSESSMENT QUESTIONS:

1) Explain in detail about dry ashing method and advantages and disadvantages of this method
2) Write about wet ashing.
3) Discuss in detail about microwave ashing methods
4) Write about the principle, procedure involved in calcium determination
5) How to determine phosphorus content?
6) Explain about determination of iron
7) What are the various methods used in analysis of minerals
8) What are the various methods for determination of vitamins
9) Discuss about the determination of vitamin A
10) Explain in detail about analysis of β-carotene
11) What are the methods used for the determination of riboflavin
12) Write an account on various analytical methods for determination of vitamin C

16.8 REFERENCE BOOKS:

1) Food analysis by S.Suzanne neilsen.
2) Food analysis Theory and practice edited by Y. Pomeranz.
3) A first course in food analysis by A.Y.Sathe.
4) Chemical analysis of food: Techniques and applications by Yolanda Pico.
5) Methods in food analysis: Edited by Rui M.S.Cruz, Igor Khmelinskii, Margarida C. Vieira.
6) Vitamins in animal and human nutrition Lee Russell Mcdowell, second edition.

7) Hand book of Food Analysis Physical Characterization and Nutrient Analysis, Leo M.L. Nollet, Second edition.

8) A first course in food analysis by A.Y.Sathe.

9) Handbook of food science technology and engineering, editor Y.H.Hui, 4 volume set.

10) Modern food analysis by F.Leslie Hart A.M. and Harry Johnstone Fisher.

11) https://people.umass.edu/~mcclemen/581Ash&Minerals.html

Dr. P. Kiranmayi

LESSON-17

BASIC PRINCIPLES AND APPLICATIONS OF SPECTROSCOPY, UV AND UV-VISIBLE SPECTROSCOPY

17.0 OBJECTIVES:

After reading this chapter, students will be able:

- To equip learners with the analytical techniques of UV-Vis spectroscopy techniques for qualitative and quantitative analysis associated with food.

STRUCTURE:

17.1 INTRODUCTION:

"The study of interaction between matter and electromagnetic radiations is known as spectroscopy". Energy from the light source will interact with the matter (may be ions, molecules or atoms).

Based on energy, frequency and intensity, the spectra of electromagnetic radiation yield radiations of varied wavelengths. High energy radiations are categorized in to below types

1) Short wavelength
2) High Frequency

Low energy radiations are given below:

1) Long wavelengths
2) Low frequency

The nature of Interaction between matter and radiation includes below properties:

1) Absorption
2) Emission
3) Scattering

The energy derived from the EMR is absorbed by the electrons of the molecule to reach the excited state by giving an **absorption spectrum** and the wavelength at which higher energy is absorbed is known as **absorption maxima**. Additionally, the source of radiation causes the energy transitions in the matter itself, where the electrons fall back from higher energy excited state to lower energy ground state by proving an **emission spectrum**. Both absorption and emission spectrum is characteristic of the wavelength of EMR and the material involved.

Spectroscopy techniques are of two types:

Spectroscopy is a technique that uses the interaction of light with matter to analyze the composition and properties of substances. There are two main types of spectroscopy:

Qualitative Spectroscopy: This type of spectroscopy focuses on identifying the components of a substance by analyzing the wavelengths of light that are absorbed or emitted by the sample. Each element or molecule has a unique spectral fingerprint, allowing for its identification.

Quantitative Spectroscopy: This type of spectroscopy measures the amount of light absorbed or emitted by a substance to determine the concentration of its components. The intensity of the spectral signal is proportional to the amount of the substance present, enabling quantitative analysis.

17.2 UV-SPECTROSCOPY:

- UV region: This refers to ultraviolet light, which has a wavelength between 200 and 400 nanometers.
- Molecules: These are groups of atoms bonded together.
- Electrons: These are tiny particles that orbit the nucleus of an atom.
- Ground state: This is the lowest energy state of an electron.
- Excitation: This is the process of an electron moving to a higher energy state.

In absorption spectrophotometry, a beam of UV light is passed through a sample of a chemical substance. Molecules in the sample absorb some of the light, and the amount of light absorbed is measured. This information can be used to identify the substance and determine its concentration.

17.2.1. Principle of UV Spectroscopy:

1) π-electrons or nonbonding electrons (n-electrons)are seen in molecules which absorb energies in UV. This excites high anti bonding orbitals.
2) Excited electrons absorbs longer wavelengths and four transitions are seen as (π–π*, n–π*, σ–σ*, and n–σ*). The order is σ–σ* > n–σ* > π–π* > n–π*
3) A distinct and specific spectrum is observed when chemical compounds absorb light in UV range. This supports the system to identify the compound later.

17.2.2. Instrumentation or Parts of UV Spectroscopy:

Light Source: The suitable light sources that covers UV region are

Tungsten filament lamp: These are rich in red radiations and emit radiations at 375 nm.

Hydrogen-Deuterium lamp: Their intensity falls below 375 nm.

Monochromator:

- A monochromator is a device used in spectrophotometers to isolate a specific wavelength of light.
- It typically uses prisms and slits to achieve this.
- In the described setup, the monochromator separates electromagnetic radiation, and specifically, a rotating prism acts as the primary radiation source.
- The resulting monochromatic beam (light of a single wavelength) is then further refined by another prism and focused through a slit.
- The mention of "double beam spectrophotometers" suggests this monochromator setup is part of a system that splits the light path for comparison with a reference sample.

Sample and Reference Cells:

- A light beam is split into two, one beam passes through the sample solution, while the other passes through a reference solution.
- Both solutions are held within cuvettes or cells, which are constructed from silica or quartz.
- Glass is unsuitable for these cells because it absorbs ultraviolet light, interfering with measurements in that region of the spectrum.

Detector:

- These have photocells to detect UV spectroscopy
- Photocells are two in number, one receives beam from sample cell and another receives from reference cell
- Intensity of reference cell is more than sample cell
- These gives pulsating and alternate currents in photocells

Amplifier:

- The alternating current from the photocells is sent to an amplifier.
- This amplifier, connected to a small servomotor, boosts the signal.
- Because the current produced by the photocells is typically very weak, amplification is crucial for obtaining clear and measurable signals.

Recording Devices:

Here the amplifier is coupled to the pen recorder and is connected to the computer. It stores all the data and gives a spectrum of desired compound.

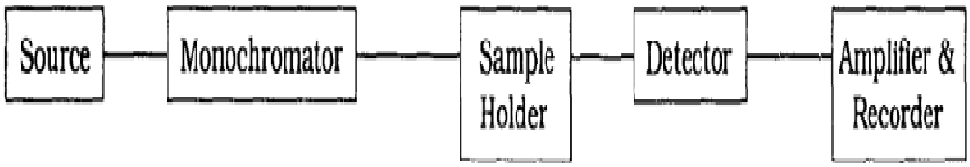


Figure 17.1 Block Diagram of a Spectrometer

17.2.3. Applications of UV Spectroscopy:

1) It can characterize UV absorbing compounds and supports qualitative determination
2) By comparing absorption spectrum, compounds are identified
3) The study of kinetics of chemical reaction can be forwarded
4) Drugs assays at different concentrations can be done
5) Molecular weights also can be determined
6) It is used as detector in HPLC
7) Impurities in organic materials also can be determined
8) It elucidates the structure of organic molecules
9) The presence of unsaturation can be checked along with heteroatoms

17.2.4. Applications in Analysis of Foods:

- UV-Visible spectroscopy is commonly used to quantify pigments such as chlorophyll, carotenoids and anthocyanins in fruits, vegetables, and beverages.
- **Measurement of enzyme activity**: Enzymes in food products can exhibit characteristic absorption spectra, allowing their activity to be monitored and quantified using UV-Visible spectroscopy.
- **Analysis of additives:** UV-Visible spectroscopy can be used to detect and quantify additives such as preservatives, colorants, and antioxidants in food products.

- **Assessment of food quality:** Changes in the UV-Visible absorption spectra of food components can indicate alterations in quality attributes such as freshness, ripeness, and shelf-life.
- **Significance:** UV-Visible spectroscopy offers several advantages for food analysis, including rapid analysis, simplicity, and versatility. It allows for the non-destructive measurement of samples, requiring minimal sample preparation. Additionally, UV-Visible spectroscopy is cost effective and can be easily implemented in food quality control laboratories.

17.3 UV- VISIBLE SPECTROSCOPY:

Ultraviolet-visible (UV-Vis) spectroscopy, also known as UV-Vis spectrophotometry "is an absorption spectroscopy technique that analyzes substances in the ultraviolet and visible regions of the electromagnetic spectrum".

These are named as follows:

1) UV-Vis Spectroscopy
2) Ultraviolet-visible Spectrophotometer
3) UV-Visible Photometer

This cost-effective, simple, versatile, and non-destructive method allows for repeated sample analysis. It involves below techniques

1) Qualitative
2) Quantitative
3) Analytical techniques

UV-Vis spectroscopy compares a sample to a blank or reference, measuring the amount of UV and visible light absorbed or transmitted. This measurement is based on the Beer-Lambert Law.

Table 17.1 Wavelengths for Different Electromagnetic Radiations

Name of the Region	Wavelengths
UV - Vis spectroscopy	190 nm to 800 nm
UV region	190 to 400 nm
Visible region	400 to 800 nm
Near UV region	190 nm to 400 nm
Far UV region	Below 200 nm

High frequency and energy are seen in the UV region of shorter wavelengths. Higher wavelengths have lower frequency and less energy seen in visible regions.

Factors Affecting Absorbance:

1) Sample composition
2) Sample concentration
3) Identification process
4) Assessing method for purity
5) Quantification of components
6) Pattern of absorption
7) Transmission of light
8) Application of Monolithic solids
9) Application of liquids, glass, powders, and thin films

Absorbance (A):

It is called optical density (OD). It is the amount of light absorbed by the object.

Absorbance (A)= -log(T)

Transmittance (T)

It is the division of intensity of spectrum of light transmitted through sample (I) by the intensity of the spectrum light transmitted through blank (I0)

T= *I/I*o

17.3.1. Principle:

The principle behind UV-Vis spectroscopy is based on the interaction of light with molecules. When a molecule absorbs light of a specific wavelength within the ultraviolet-visible range (200-800 nm), its electrons transition from their ground state (lower energy level) to an excited state (higher energy level). Because these electronic transitions occur between discrete, quantized energy levels, only specific wavelengths of light are absorbed.

The Beer-Lambert Law equation is the principle behind absorbance spectroscopy. By using this, the following can be determined

1) Concentration of sample
2) Absorption spectra Patterns
3) Wavelength relation with transmittance

Beer-Lambert Law:

It was formulated by a German Mathematician, chemist named August Beer in 1852. According to him, the absorptive capacity of dissolved substance is directly proportional to concentration of solution.

$$A = \varepsilon lc$$

- Where *A* is absorbance
- ε is the molar extinction coefficient
- *l* is the length of the path light must travel in the solution in centimeters,
- *c* is the concentration of a given solution
- When absorbance of series of samples are plotted against equivalent concentrations, calibration graph can be obtained which is linear

17.3.2. Instrumentation of UV-Vis Spectroscopy:

The major segments of UV-Vis spectrophotometer are given below

1) Source of light
2) Selector for wavelength
3) Container for sample
4) Detectors

1. Source of Light:

Tungsten filament lamps are used as a light source. These are used in visible regions. Hydrogen and deuterium lamps are also used. Both lamps are switched on and it depends on the user. Mechanical switch directs the light source.

2. Selector for Wavelength:

There is a monochromator which gives light of single colour or single wavelength which is passed through samples. It is an optical instrument that selects light of single wavelengths from a wide range. The phenomenon of diffraction is seen along with prisms. Gratings are used for diffraction. Prisms show higher dispersion in the UV region. These contain collimators that convert diverging light to collimated light

Single-beam and double beam spectrophotometer:

In a single beam, radiation comes from a light source and passes through a sample. A beam that determines colour by comparing light intensities is seen. It can measure upto 1100 nm. In a double beam, radiation slits from two beams, one through sample and other through reference. Wavelengths of 190 to 1100 nm are operated. It measures absorbance vs wavelength and sample vs beam ratio.

3. Container for Sample:

Sample should be placed in a transparent cuvette, which is in rectangular shape. This will have an internal width of 1cm. small test tubes are used. Cuvettes are made of silica. These will detect in visible range. Borosilicate, fused silica and quartz cuvettes are used.

The light passes through the beam separator to the reference chamber and sample. Intensity of light measures the number of photons per second. The sample that do not absorb light is seen in blank sample. If sample I is less than Io, the sample absorbed some light.

The absorbance (A) of the sample is related to I and Io and are given as below

Absorbance (A) = - log (T) = - log (*I*/*I*o)

This equation shows the relationships between absorbance and transmittance. Also, the fraction *I* divided by *I*o is called transmittance (*T*), which expresses how much light has passed through a sample.

$$T = I / Io$$

$$T = I / Io = e - kbc$$

Where:

- Io is the incident intensity
- I is the transmitted intensity
- e is the base of natural logarithms
- k is a constant
- b is the path length (usually in cms).

The lighter the refracted, the more transmittance occurs. Lower the absorbance, the higher the transmittance.

4. Detectors:

These rely on semiconductors. Incoming light is converted to current. More current gives greater intensity. A variety of wavelengths and sensitivities are seen. Absorbance against wavelength in the UV and visible section is plotted by a data recorder**.**

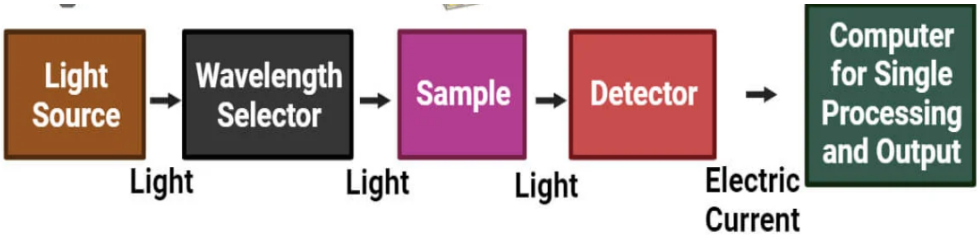


Fig.: 17.2 Instrumentation of UV-Vis Spectroscopy

17.3.3. Applications of UV-Vis Spectroscopy:

DNA and RNA analysis

1) Downstream applications
2) DNA and RNA purity check
3) Sequencing of DNA and RNA
4) Pure DNA absorbance ratio of 1.8and RNA is of 2.0
5) Protein contamination in nucleic acids
6) Absorbance ratio check for RNA and DNA

Pharmaceutical Analysis:

1) Drug discovery
2) Drug development
3) Quantification of impurities
4) Dissolution property of tablets
5) Chemical identification
6) Chemical quantification
7) Overlapping of absorbance peaks
8) Identification of pharma compounds
9) Veterinary powder formulation

Food and Beverage Applications:

1) Sensory evaluation
2) Nutritional composition
3) Chemical composition of foods
4) Detection of contaminants
5) Detection of adulterants
6) Quality control in wine
7) Identification of anthocyanins in fruits
8) Evaluation of food and food product colour

Bacterial Culture:

1) It's a key tool for generating biomass growth curves by measuring the optical density of cultures at 600nm.
2) This measurement allows for the estimation of cell concentrations and the tracking of bacterial growth.
3) The 600nm wavelength is specifically chosen to minimize interference with the culture media's optical properties and to prevent cell damage, which is especially important for continuous experiments**.**

Other Applications:

1) Used to evaluate photostability agents and colour index in cosmetics
2) Quantification of dyes and antioxidants
3) Detection of adulterants

4) Characterization of nanoparticles in material sciences
5) Determination of battery composition
6) Examines structural protein changes
7) Waste water treatment procedures
8) Monitoring studies of dyes
9) Dye removal processes
10) Measurement of colour index
11) Used in petrochemistry for characterizing crude oils
12) Quality checking of crude oil gravity
13) Indices formulation for aromatic content and sulphur content
14) Quantification of DNA, proteins, Enzymes, thermal denaturation of enzymes.

17.4 SUMMARY:

UV and UV-Vis spectroscopy are versatile and widely used techniques. These instruments provide valuable information about the composition and properties of substances. The Beer-Lambert law is fundamental to quantitative analysis using UV and UV-Vis spectroscopy. The applications of UV and UV-Vis spectroscopy are diverse and span many scientific disciplines.

17.5 TECHNICAL TERMS:

UV Spectroscopy, UV-Visible Spectroscopy Hydrogen-Deuterium lamp, Monochromator, Amplifier, Beer-Lambert Law

17.6 SELF-ASSESSMENT QUESTIONS:

1) Write about principle and applications of UV Spectroscopy
2) Explain the applications of UV-Vis Spectroscopy

17.7 SUGGESTED READINGS:

1) Silverstein, R.M., Webster, F.X., & Kiemle, D.J. (2005). *Spectrometric identification of organic compounds.* Hoboken, NJ: John Wiley & Sons.
2) Biophysical chemistry Principles and techniques *D.D. Upadyaya and Nath.*

Prof. J. Rajeswari

LESSON-18

BASIC PRINCIPLES AND APPLICATIONS OF AAS, AES, ELECTROMAGNETIC RESONANCE

18.0 OBJECTIVES:

After reading this chapter, students will be able to:

- To equip learners with the analytical techniques of atomic absorption spectroscopy, atomic emission spectroscopy, and electromagnetic resonance spectroscopy techniques for qualitative and quantitative analysis associated with food.

STRUCTURE:

18.1 INTRODUCTION:

Atomic absorption Spectrophotometry is a popular analytical method that measures how much electromagnetic radiation is absorbed by gaseous atoms.

In order to vaporize the sample, atomic absorption spectrophotometers are sometimes fitted with a flame burner, usually a hollow cathode lamp. These devices also come with a monochromator and a photon detector.

Numerous samples, including soils, water bodies like lakes, rivers, and oceans, as well as potable water sources, pharmaceuticals, food and beverage items, geological and mineralogical specimens, petroleum derivatives, biological fluids and samples, and forensic investigations, can be examined for trace metals using this technique.

18.2 ATOMIC ABSORPTION SPECTROPHOTOMETRY (AAS):

This method is used to measure the amount of metallic elements present in the sample. This technique is thought to be one of the best ways to detect minuscule amounts of a sample, including those that are present at one part per billion (ppb) levels. The process involves a group of unbound atoms absorbing radiant radiation, usually visible and ultraviolet. The used specimen is in a gaseous state.

Electrons are excited from their ground state to the excited state when an atom absorbs light of a specific wavelength. Both qualitative and quantitative evaluations can use the constant amount of energy absorbed per mole.

In order to identify the constituents of interest, the technique uses particular electromagnetic radiation wavelengths that are released from a light source. By comparing the absorbance of each element to predetermined standards, one can ascertain how differently each element absorbs wavelengths. The unique light wavelengths that are selectively absorbed by individual atoms are used by the atomic absorption spectroscopy technique.

18.2.1 Principle of Atomic Absorption Spectrophotometry:

The analyte's constituent atoms have an unchanging and consistent number of electrons. Electrons in different energy levels inside their own orbitals are excited to higher energy levels by absorbing a certain wavelength of light when a flame containing a particular atom is exposed to light at that wavelength. There exists a direct proportionality between the amount of ground-state atoms in the flame and the level of absorption.

Compared to the higher energy levels, also called excited states, the ground state, which is the lowest energy state, is more stable. There is a spontaneous propensity for the electrons to go back to their ground state. An equivalent amount of radiant energy is released by it. The term fluorescence is frequently used to describe the phenomena. Fluorescence is used as a method in atomic emission spectroscopy.

For instance, the sodium (Na) atom experiences excitation of its outermost electrons to higher energy levels upon absorption of radiation with a wavelength of 589 nm. It then reverts to its ground condition, or starting state. It's crucial to remember that during the de-excitation process, the emission at the 589 nm wavelength stays constant.

The Boltzmann equation provides an explanation for the population of ground and excited states.

$$N_1/N_o = (g_1/g_0)\, e^{\Delta E/KT}$$

Where,

- N_1 is the number of atoms in the excited state.
- N_o is the number of atoms in the ground state.

- g_1/g_o is the ratio of statistical weights for ground and excited states.
- ΔE is the energy of excitation ($=h\vee$).
- k = Boltzmann constant.
- T=temperature in Kelvin

18.2.2 Instrumentation of Atomic Absorption Spectrophotometry:

The fundamental tools used in atomic absorption spectroscopy.

Source of light: It is discovered that a hollow cathode lamp provides the light source. A cylindrical hollow tube made of the same cathode-acting element makes up the cathode lamp. The substance that makes up the anode is tungsten. There is a glass tube with a quartz window that houses the anode and cathode.

Inactive gases, specifically argon and neon, are charged inside the glass enclosure at a reduced ambient pressure of 1 to 5 torr. The noble gases at the anode are ionized when a high voltage (between 300 and 500 V) is applied to the electrodes. The resultant ions are driven in the direction of the cathode. Some metal atoms sputter when the high-velocity ions collide with the cathode surface. When metals are evaporated, electrons are excited to higher energy levels. This effect can be explained by the continuous interaction with gas ions that have higher energy levels.

Chopper: A chopper is a rotating apparatus that is placed between a flame and a hollow cathode lamp. Macroscopic droplets are removed during the process, and droplets with uniform sizes are allowed to enter the combustion zone. The constant light from the bulb is modified into a pulsating shape. Without any known interference, the photocell's pulsing current is amplified and recorded.

System for introducing samples: This device is used to move samples to the atomizer. Ideally, there should be no interference, total independence from the type of sample, and universal compatibility with all atomizers. One commonly used tool for introducing solutions is a pneumatic nebulizer. A compressed air jet that also aspirates and nebulizes the nebulization gas is used to nebulize the solution through a capillary tube.

Nebulizers: Concentrated nebulizers and angled or cross flow nebulizers are the two types of nebulizers that are most commonly utilized.

Atomizer: There are two different ways to carry out the atomization process: using a furnace or a flame. Heat energy is used in atomic absorption spectroscopy to convert metallic elements into atomic dissociated vapor. The effective conversion of atomic vapor depends on precise temperature control. Atoms may undergo the ionization process at high temperatures. After entering a mixing chamber, the fuel and oxidant gasses pass via baffles and eventually arrive at the burner. A ribbon-shaped flame is produced by the absorption spectrophotometry equipment. Aspiration is used to introduce the specimen into the mixing chamber.

Monochromators: An optical device known as a monochromator selectively transmits a limited range of light or radiation wavelengths from a wider spectrum. The device known as the Atomic Absorption Spectrophotometer (AAS) makes it easier for the atoms that make up the object to absorb energy, which in turn causes radiation to be released. By transmitting a small band, a monochromator can be used to isolate a desired band of lines.

Monochromators come in two varieties: diffraction gratings and prisms. In comparison to the dispersion created by a prism, the dispersion displayed by a grating is more uniform in nature. Because of its wide range of wavelength resolution, the grating can operate continuously for an extended amount of time.

Detectors: A detector can be used to transform light coming from a monochromator into a simpler electrical signal. The detector of the device used for atomic absorption spectrophotometry is usually a photomultiplier tube. It is possible to modify a detector such that it responds to a specific wavelength or frequency.

Amplifiers: After the output of the detectors is sent to amplifiers, the current's magnitude is increased by several orders of magnitude.

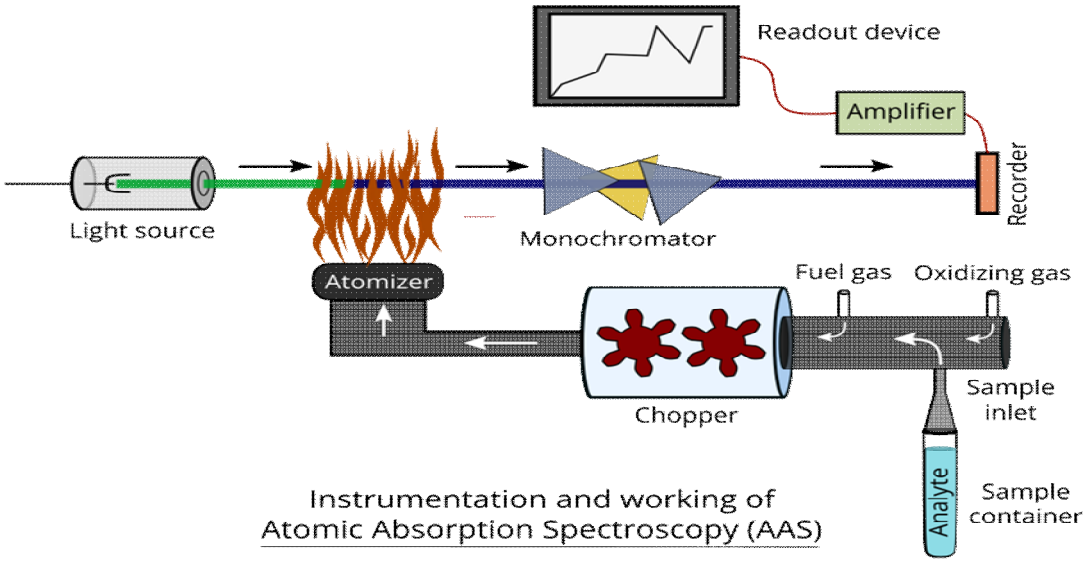


Fig.: 18.1: Instrumentation of AAS

Read out Devices:

Atomic absorption spectroscopy uses a variety of readout technologies, such as digital display meters, chart recorders, and meters. Plotters, printers, and chart recorders can all be used to create hard copies.

18.2.3 Applications of Atomic Absorption Spectrophotometry:

- **Mining and Geology:** Determining the elemental makeup of rocks and minerals is essential for evaluating the profitability of mining activities in areas that have been studied. To guarantee the maximum effectiveness of the refining processes, it is essential to do a compositional analysis on the extracted ores and minerals after the extraction process. Exploration of water and oil reservoirs greatly benefits from trace metal analysis.

- **Environmental monitoring:** Monitoring environmental conditions for trace metal pollution in rivers, lakes, oceans, and industrial discharges is essential for assessing whether water is suitable for drinking and commercial use. Determining whether these samples are within the safety ranges that regulatory bodies have established is crucial. The evaluation and feasibility of a site for business establishments are significantly impacted by environmental monitoring.
- **Pharmaceuticals**: Establishing dose limits, determining catalyst performance, and developing formulations all depend heavily on the study of trace metals using atomic absorption spectrophotometry. The majority of elements have beneficial characteristics within certain suggested limits, however exceeding those limits might have negative effects.
- **Oil and petroleum**: Both mineral oils and edible oils must undergo refining before being consumed. Both distillation and catalytic refining may be used in the refining process. Metal absorption during these activities could lead to a decline in performance or possibly endanger customers. Analyzing engine oil for trace metals can provide important diagnostic information about how engine parts deteriorate.
- **Material development**: The composition of materials and the presence of trace metals can significantly alter their physical properties, such as their hardness, brittleness, grain size, crystallinity and amorphous nature. Atomic absorption spectrometry study of trace metals can provide important information on the performance properties of certain materials.
- **Food and beverages**: The application of this method is common in the food and beverage industry to assess the levels of various components in wine, beer, and fruit-based drinks. One method used to identify the many types of contaminants found in food is atomic absorption spectrometry. Iron, manganese, copper, zinc, mercury, lead, and nickel can all be detected in human urine and blood.
- **Forensic**: It provides important information about specimens such as stomach contents used in foodborne illness investigations, paint chips, fibers, and hair strands collected at the scene of a crime.
- **Agriculture**: The identification and measurement of metallic components, such as calcium, strontium, and cobalt, in soil samples is accomplished by the use of atomic absorption spectrophotometry. Additionally, it is used in animal feed analysis to find out which elements-such as Zn, Cu, Mn, Ca, K, and Na-are present. In order to determine the concentrations of different elements, such as Ca, Cu, Mg, and Fe, this method is used to analyze fertilizer that comes from animals.

- Applying this technique is essential when heavy metal toxicity is present. Because heavy metal poisoning can be fatal, it is crucial to regularly check the patient's blood levels for poisoning.
- It is crucial to check for metals in groundwater and bore well samples before drinking them or using them for irrigation.

18.3 ATOMIC EMISSION SPECTROSCOPY (AES):

The analytical method known as “atomic emission spectroscopy” (AES) measures the amount of light that metal atoms emit when they are stimulated. An excited atom releases a certain wavelength of radiation upon returning to its ground state. Atomic emission spectroscopy involves the excitation (absorption of radiation) and de-excitation (emission of radiation) of electrons.

In order to determine the structure, composition, and environment of atoms, atomic emission spectroscopy examines the radiation that they emit. Theories of atomic structure have an experimental foundation since wavelength measurements allow us to determine the atom's energy levels, also known as stationary states.

18.3.1 Principle of Atomic Emission Spectroscopy:

AES is based on the idea that when energy is delivered to a molecule in the form of light or heat, molecules are excited and shift from a lower energy level state to a higher energy level state. The molecules are unstable at higher energy levels and revert to lower energy levels after producing radiations in the form of photons. The emission spectrometer measures the wavelengths of emitted photons. The basic principle of atomic emission spectroscopy is the study of the wavelengths of photons released by atoms and molecules as they move from a high-energy state to a low-energy state. Each element or substance emits a distinct set of wavelengths that are determined by its electrical structure. The elemental structure of the sample can be revealed by studying wavelengths.

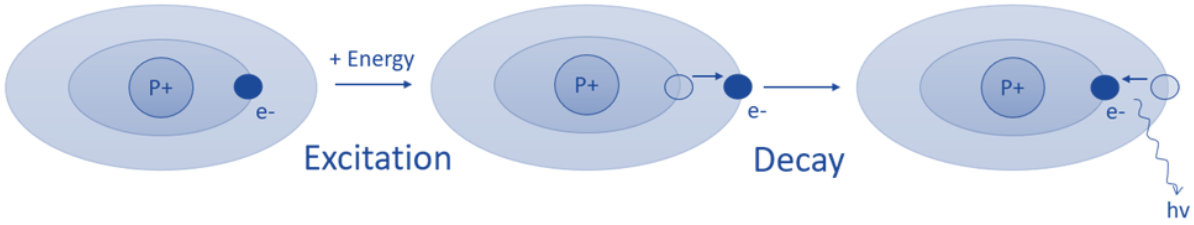


Fig. 18.2: Basic Principle of Atomic Emission Spectroscopy

18.3.2 Instrumentation of Atomic Emission Spectroscopy:

Nebulizer:

The goal is to convert the gaseous sample into highly excited free atoms. Using a nebulizer to create aerosol is the most practical method of turning liquids into a gas stream. The movement of a fast-moving jet across the narrow opening's tip might produce the aerosol. Droplet sizes have a significant impact on the stability of the spectrum emission. Therefore, selecting the appropriate nebulizer type is essential to achieving uniform droplet sizes. Sample characteristics include density, viscosity, organic content, total dissolved solids, and total sample volume determine which nebulizer is best.

Excitation Sources:

An excitation source is used to dissolve, atomize, and excite the sample's atoms. All of the elements in the sample will be able to be excited by the perfect excitation source, which will continue to do so until all of the elements in the sample have been excited.

Direct-Current Plasma:

This excitation technique heats the plasma gas, which is typically argon, by creating an electrical discharge with two electrodes. Materials with a high solids content are more suitable for this type of stimulation.

Inductively Coupled Plasma:

The most popular excitation technique involves generating excitement in the material using a plasma torch composed of concentric quartz tubes. A high-frequency inductively coupled plasma serves as the light source for inductively coupled plasma atomic emission spectroscopy (ICP-AES).Wavelengths used by AES range from visible light (800 nm) to the upper vacuum ultraviolet (160 nm). It involves using radiation absorption to excite and de-excite electrons.

When an electron moves from an excited to a de-excited state, electromagnetic radiation (EMR) is measured and assessed. The term optical emission spectroscopy (OES) refers to the optical quality of the radiation during the de-excitation process. Atomic emission spectroscopy allows for measurement because the spectral line has a defined wavelength.

Flame:

This process involves exposing a sprayed solution or gas that contains a sample of the material to be studied to flame. Free atoms of the material are produced when the solvent is evaporated by the flame heat and the analyte's chemical bonds are broken. Additionally, heat transforms atoms into electrically charged particles, which, when returning to their ground electronic state, emit light. The idea behind a flame photometer is to measure the amount of light that is released when a metal is added to a flame. The flame's color reveals the amount of elements in the sample, while the color's wavelength identifies the element.

Microwave Induced Plasma (MIP):

A microwave induced plasma (MIP) source uses frequencies in the microwave area as an external energy source. Usually, microwave radiation with a frequency of 2450 MHz is used. A microwave-induced plasma is created when ultrahigh frequency ac power is capacitively linked into a stream of nitrogen or noble gas (argon or helium) at a rate of about 3 dm3/min in a resonant cavity.

Laser-Induced Plasma (LIP):

In this process, a high-energy CO2 laser source focuses a support gas, usually argon, to maintain the heated plasma.

Arc or Spark:

Spark and arc excitation sources evaporate and excite the atoms in the sample using a spark, an electric pulse, or a continuous electrical discharge arc between two electrodes.

Monochromator:

Prisms and diffraction gratings are examples of monochromators. These are employed to eliminate any further unwanted radiation and to choose the particular type of radiation produced by the analyte. It is therefore also referred to as "the wavelength selector." Compared to prisms, diffraction gratings yield more resolution and precision.

In the AES spectrometer, gratings are used as a dispersive element to break up incident light into its component wavelengths. At wavelength-dependent diffraction angles, constructive interference distributes wavelengths as a result of the light being reflected off the angled grating surface. A polychromator with several detectors can detect atoms in a sample concurrently or sequentially with a monochromator since all of the atoms are excited simultaneously.

Detectors:

Detectors are transducers that transform the analog output of the spectrometer into an electric signal that a computer can view and process. Photomultiplier tubes (PMTs), charge-coupled devices (CCDs), and charge-injection devices (CIDs) are examples of detectors used in atomic emission spectroscopy. The amplifier then boosts the electrical current that they have converted from optical impulses.

Amplifier:

After receiving signals from the detectors, the amplifiers multiply them several times to make them similar or usable.

Readout Device:

Computers are used as readout devices for atomic emission spectroscopy. Computers examine the data in the form of spectra and plot the calibration curve using the atomic emission ranges library.

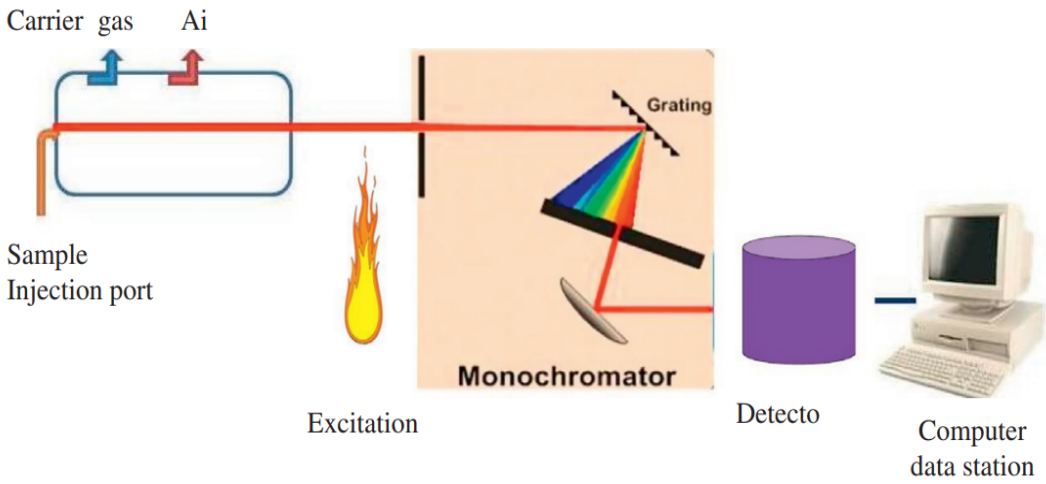


Fig. 18.3: Atomic Emission Spectroscopy

18.3.3 Applications of Atomic Emission Spectroscopy:

i) Food and agricultural products can be analyzed in agriculture using the ICP-AES method.

ii) Rare earth elements found in rocks can be analyzed using it in earth science.

iii) Traces of metals from alloys, steel, lubricants, and gasoline can be examined using the ICP-AES technique.

iv) In biology, the ICP-AES method can assess the levels of copper in brain tissue, selenium in liver, aluminum in blood, and salt in breast milk.

v) Metal traces in beer or wine, including calcium (Ca), copper (Cu), iron (Fe), manganese (Mn), magnesium (Mg), phosphorus (P), potassium (K), and zinc (Zn), can be found using inductively coupled plasma atomic emission spectroscopy.

vi) The human body needs certain amounts of Na+ and K+ ions to carry out a variety of metabolic functions. A blood serum sample can be diluted and aspirated into the flame to determine the amounts of these chemicals.

vii) The levels of different metals and elements in fruit juices, soft drinks, and alcoholic beverages can be evaluated using flame photometry.

viii) Flame photometry can also be used to measure the concentrations of different metals and elements in fruit juices, soft drinks, and alcoholic beverages.

ix) Calcium and magnesium levels in cement are determined using AES.

x) Lead in gasoline is detected using AES.

xi) AES was used to assess the amounts of Ca, Mg, Na, and K in blood serum and plasma.

xii) AES is a useful technique for identifying metallic pollutants.

xiii) AES is able to identify the soil's metallic content.

xiv) Petroleum product adulteration can be identified using AES.

18.4 BASIC PRINCIPLES AND APPLICATIONS OF ELECTROMAGNETIC RESONANCE:

A large class of analytical methods known as electromagnetic resonance spectroscopy examines the structure, behavior, and characteristics of matter by studying how electromagnetic radiation interacts with it. Nuclear Magnetic Resonance (NMR), Electron Paramagnetic Resonance (EPR), and a number of additional techniques are used in this discipline.

Basic Principles of Electromagnetic Resonance Spectroscopy:

1) **Energy Absorption and Resonance**: Transitions between distinct energy levels occur when electromagnetic radiation of a certain frequency is absorbed by atoms, nuclei, or molecules.

   According to the formula,

   ΔE=hν\Delta E = h \nuΔE=hν,

   Where h is Planck's constant and ν\nuν is the radiation frequency, the absorbed energy must equal the energy gap (ΔE\Delta EΔE) between these levels.

2) **Magnetic Field Influence**: Numerous methods, including NMR and EPR, split energy levels using the Zeeman effect and an external magnetic field, making it possible to investigate the spin characteristics of electrons or nuclei.

3) **Relaxation Processes**: Energy is recognized and assessed when the system relaxes after excitation to revert to its lower energy state.

4) **Spectroscopic Information**: The sample's electrical, vibrational or rotational states can be inferred from the location, strength, and form of the absorption or emission peaks.

General Applications of Electromagnetic Resonance Spectroscopy:

1) **Structural Analysis**:

   - Identifying the crystal and molecular structures.
   - Chemical bonds and functional groupings are identified.

2) **Biological Research**:

- DNA and protein research (e.g., NMR spectroscopy for protein dynamics and folding).
- Free radical detection in biological systems (e.g., EPR).

3) **Material Science**:

- Catalyst, polymer, and semiconductor characterization.
- Investigation of electrical and magnetic characteristics.

4) **Environmental Studies**:

- Examination of toxins and pollutants.
- Identification of species and trace components in the atmosphere.

5) **Medicine**:

- Imaging that is non-invasive (MRI based on NMR principles).
- Study of the metabolic mechanisms underlying disease.

18.5 SUMMARY:

The atomic absorption spectrophotometer's principles, which underpin AAS and AES in food analysis, are essential for guaranteeing the quality, safety, and authenticity of food products. AAS and AES are flexible instruments that enable the food sector to satisfy consumer needs for dependability and openness, from tracking vital nutrients to identifying dangerous chemicals. AAS in food analysis will develop as technology advances, providing even more speed and accuracy in elemental analysis.

18.6 TECHNICAL TERMS:

Atomic Absorption, Atomic Emission, Electromagnetic Resonance, Spectroscopy, Monochromator, etc.,

18.7 SELF-ASSESSMENT QUESTIONS:

1) Write about atomic absorption spectrophotometry.
2) Give principles of atomic absorption spectrophotometry.
3) Write about atomic emission spectrophotometry.
4) Write about electromagnetic resonance spectroscopy.

18.8 SUGGESTED READINGS:

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3) Filho HJI, Salazar RF, Capri M, Neto AC, de Alcantara MAK, Peixoto AL. State of the art trends in atomic absorption spectrometry. Intechopen. Published 2011.
4) Walsh, A. Atomic Absorption Spectroscopy-Stagnant or Pregnant?
5) H. H. Willard, L.L. Merritt, J.R. Dean, and F.A. Settle, *Instrumental Methods of Analysis* (7^{th} Edition), CBS Publishers and Distributions, India, 1986.
6) Vogel's Textbook of Quantitative Chemical Analysis, 6^{th} Edition, 2008.

Prof. J. Rajeswari

LESSON-19

CHROMATOGRAPHY - PRINCIPLES AND APPLICATIONS OF HPLC

19.0 OBJECTIVES:

After reading this chapter, students will be able to:

- To equip learners with the analytical techniques of high-performance liquid chromatography

STRUCTURE:

19.1 INTRODUCTION:

Analytes dissolved in the mobile phase are separated using high-performance liquid chromatography (HPLC) technology by means of a specific interaction with a stationary phase.

It is generally a much improved type of column chromatography. Instead of being allowed to pass through a column naturally, a solvent is pushed through it at high pressures. It is therefore significantly faster.

Because the column packing material has a lower particle size, there is a significantly greater surface area available for interactions between the stationary phase and the molecules passing through it. The components of the combination can also be separated much more successfully as a result. The other major improvement over column chromatography is the use of various detection methods. Additionally, these procedures are quite delicate.

19.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY:

This method uses small diameter columns (1-3mm) with support particle sizes of about 30m. The eluent is injected through the column at a high flow rate in HPLC. Research indicates that separation using HPLC can be completed about 100 times quicker than with conventional liquid chromatography. By operating at high pressures and offering analysis times comparable to GLC, these liquid chromatography systems also mitigate the impact of higher liquid viscosities in comparison to gas viscosities.

19.2.1 History and Types of HPLC:

In addition to laying the groundwork for liquid-liquid chromatography and high-performance liquid chromatography, Martin and Synge reported the discovery of liquid-liquid partition chromatography in 1941. Additionally, they developed the concept of the Height Equivalent to the Theoretical Plate, which is currently a benchmark for assessing chromatography efficacy. In traditional column liquid chromatography, gravity causes the mobile liquid phase to flow through the column slowly. Two general features of this method are long separation times and low column efficiency. Since around 1969, there has been a noteworthy resurgence of interest in liquid column chromatography technology as a result of Kirkland and Huber's discovery of HPLC.

Types of High Performance Liquid Chromatography (HPLC):

The different types of HPLC methods are as follows:

I) Based on Modes of Chromatography:

1) Normal Phase Mode:

This mode uses a column packed with silica particles and a non-polar solvent, like hexane. The mixture's non-polar compounds will pass through the column more quickly because the polar chemicals stick to the polar silica for a longer duration than non-polar ones do.

2) Reverse Phase Mode:

The column containing silica particles that have had their surface modified to become non-polar by adding long hydrocarbon chains (consisting of 8–18 C atoms) is used in reverse phase mode. Next, a combination of polar solvents, such as an alcohol-water solution, is employed. The polar compounds will flow through the column faster because of the strong attraction between the polar solvent and the polar molecules in the mixture. Hydrocarbon groups are less soluble in the components of the aqueous mobile phase, which makes it easier for the non-polar molecule to interact with them. Non-polar molecules move slowly in the column as a result.

II) Based on Principle of Separation:

1) Adsorption chromatography: This technique uses reversible binding to a stationary phase to retain analytes.
2) Ion exchange chromatography: This technique divides ions and polar substances based on how well they bind to ion exchangers.
3) Ion pair chromatography: This effective reversed-phase liquid chromatography method enables the neutralization and separation of ions in solution as ion pairs.
4) Size exclusion (also known as gel permeation chromatography): This technique divides molecules in a solution according to their size and, in certain cases, molecular weight.
5) Affinity chromatography: The method of separation in which the analyte is separated by a biospecific ligand contact.
6) Chiral phase chromatography: This kind of chromatography is used to separate the enantiomers of chiral chemicals.

III) Based on the Elution Technique:

1) Isocratic separation: It keeps the mobile composition of the mobile phase constant during the elution procedure.
2) Gradient separation: In this method, the composition of the mobile phase varies at regular intervals throughout the elution process.

D. Depending on the operational size

1) **Analytical HPLC:**

The quality and quantity of a substance are ascertained using the systems used in an analytical HPLC run. Analytical HPLC systems are used in the pharmaceutical and food safety sectors in addition to industry, healthcare, and research.

2) HPLC Preparation:

The chemical and pharmaceutical industries, as well as biotechnology and biochemistry, use preparative high-performance liquid chromatography (HPLC) to separate and purify important molecules.

IV) Based on the Type of Analysis:

1) Analysis of qualitative data

In qualitative analysis, a sample component is identified by comparing its retention period to that of a standard sample.

2) Analysis that is quantitative

A calibration curve is used to do quantitative analysis. The concentration ratio and peak area ratio are used to create a calibration curve for quantification.

19.2.2 Principles of HPLC:

The main separating principle in HPLC is adsorption. When a combination of components is put to the column, there are a variety of chemical and physical interactions between the sample molecule and the column packing particles. They move according to their individual affinities to the stationary phase. Moving more slowly is the component that is more drawn to the stationary phase. Moving more slowly is the component with a lower affinity for the adsorbent.

A tiny quantity of sample is added to a tube that is packed with microscopic particles in the HPLC process. Individual components of the sample are transported along the packed tube when a high pressure provided pump drives liquid through the column.

These components are separated by the column packing using a variety of chemical and physical interactions between their molecules and the packing particles.

19.2.3 Instrumentation of HPLC:

The Major Components of an HPLC are:

1) Solvent delivery system
2) Pumps
3) Sample injection system
4) Column
5) Detectors
6) Recorders

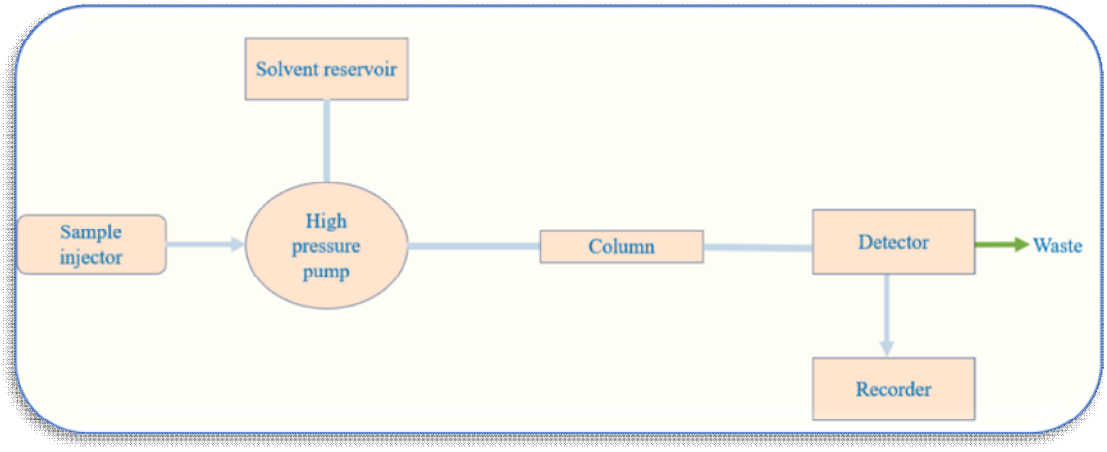

Fig. 19.1 Sample Analysis Mechanism

Solvent Delivery System (Solvent Reservoir):

The solvents or mobile phases must be forced through the column at high pressures (between 1000 and 3000 psi). Because the eluting power of the mobile phase is affected by its overall polarity, the polarity of the stationary phase, and the characteristics of the sample, choosing the right mobile phase is essential in HPLC. Because they take longer to pass through the column, more viscous solvents are generally avoided. This is because they can result in peak widening, poor resolution, and high pressure to force the solvent through the column.

The Pump System (High Pressure Pump):

The pump pushes a mobile phase through the column at a certain flow rate. Additionally, pumps provide the column with a continuous flow of the mobile phase at a fixed pressure.

Sample Injection System:

An injector is used to introduce the liquid sample into the flow stream of the mobile phase. Injectors are used to constantly inject the sample into the mobile phase stream. The injector must also be able to tolerate the high pressure in the liquid system. In a similar vein, injection needs to be repeatable and inert in order to maintain a high degree of precision.

Column:

The chromatograph's central component, the column, is often constructed of stainless steel to withstand the high pressure that the pump applies when moving the mobile phase through the column packing. Silica gel is commonly utilized as the column packing because of its weak structure, surface properties, and particle shape, which allow for efficient separation. The stationary phase of the column uses a range of physical and chemical interactions to separate the sample component of interest.

Detectors:

The detector converts information into an electrical signal by identifying the specific molecules that elute from the column. The type of analyte or sample being detected is also taken into consideration while choosing the detectors. It must be unaffected by modifications to the composition of the mobile phase.

Recorder:

The liquid chromatogram is produced by passing the electrical signal from the detector through the recorder.

19.2.4 Applications of HPLC:

HPLC is used for various purposes. Some of them are as follows:

1) There are several uses for HPLC. Here are a few of them:
2) It is employed for industrial items and fine chemicals to ensure product purity and quality.
3) Purification and separation of biopolymers, including enzymes and nucleic acids.
4) HPLC may also be used to measure the quantity of DEET in human urine.
5) Identifying endogenous neuropeptides in the brain's extracellular fluid.
6) Calculations of the shelf life of pharmaceutical products.
7) Diphenhydramine detection in sediment sample analysis.
8) Identifying anabolic steroids in serum, hair, perspiration, and urine.
9) Measurement of drugs used in psychotherapy in human plasma.
10) Sugar content analysis of fruit juices.
11) Ensuring soft drink quality and consistency.
12) Ion exchange HPLC is the most effective method for separating ionic amino acids.
13) Additionally, it is also used for the analysis of polycyclic aromatic hydrocarbons in vegetables and fruits.

19.3 SUMMARY:

The first step in HPLC is injecting the sample onto the column. After that, the mobile phase moves the sample through the packed column, where longitudinal diffusion and band broadening processes separate the compounds according to their distinct chemical characteristics. The next step is detection, when each separated compound's elution peak is recorded by the system. The concentration of each chemical in the sample is then calculated using the area under each peak.

19.4 TECHNICAL TERMS:

High-performance liquid chromatography, column, enzymes, distillates.

19.5 SELF-ASSESSMENT QUESTIONS:

1) Give working principle of HPLC

2) Write about the instrumentation of HPLC

3) Give Applications of HPLC

19.6 SUGGESTED READINGS:

1) E. Heftmann 2004. Chromatography, Sixth Edition: Fundamentals and Applications of Chromatography and Related Differential Migration Methods-Part B: Applications

Prof. J. Rajeswari

LESSON-20

PRINCIPLES AND APPLICATIONS OF GC/MS AND LC/MS

20.0 OBJECTIVES:

After reading this chapter, students will be able to:

- To equip learners with the analytical techniques of high-performance liquid chromatography

STRUCTURE:

20.1 INTRODUCTION:

In Gas Chromatography (GC), a sample is volatilized and then carried down a coated glass capillary column using an inert gas. The interior of the column is covered by the "stationary phase". The "retention time", or how long it takes a certain chemical to move through the column and arrive at the detector, can be used to identify the molecule when compared to a reference. During the conventional Mass Spectrometry (MS) step of GC/MS, compounds exiting the GC column are broken apart by electron impact. Once the charged fragments have been located, the identity of the molecule can be ascertained using the ensuing spectrum. Reproducible fragmentation patterns can be used to provide quantitative measurements.

20.1.1 Gas Chromatography:

For smaller volatile and semi-volatile chemical compounds, including pesticides, steroids, fatty acids, hormones, and hydrocarbons, alcohols, and aromatics, GC-MS is the preferred separation method. When combined with the detection power of a mass spectrometer (MS), GC can be used to separate complex mixtures, quantify analytes, identify unknown peaks, and determine trace contamination levels.

To identify compounds, Gas Chromatography-Mass Spectrometry (GC-MS) combines two advanced techniques. GC-MS analyses are often restricted to volatile and semi-volatile chemicals, while they can be used to solid, gaseous, and liquid samples. Gas chromatography is used to separate the components of a mixture, while mass spectroscopy is used to characterize each component independently. By integrating qualitative and quantitative methods, an analytical chemist can evaluate a solution made up of several compounds.

20.1.2 Principle of Gas Chromatography-Mass Spectrometry (GC-MS):

The hyphenated analytical technique is gas chromatography-mass spectrometry (GC-MS). From the end of the column to the entrance to the MS ion source, where chemicals that elute from the column are converted into ions, a heated transfer line connects the instruments. It is feasible to separate, identify, and quantify complicated chemical combinations. It is therefore ideal for examining the numerous relatively low-molecular-weight compounds found in biological and environmental materials. Before the analytes are separated using the gas-phase separation method, or GC, they must be volatilized. For a molecule to be evaluated by Gas Chromatography-Mass Spectrometry, it must be sufficiently volatile and thermally stable.

Heat extraction and pyrolysis procedures are also commonly used in geochemical investigations to study solid samples; while more frequently combined with GC-FID, they can also be paired with GC-MS for characterizing the evolved organic fractions.

20.1.3 Instrumentation of Gas Chromatography-Mass Spectrometry (GC-MS):

A Gas Chromatography-Mass Spectrometry system consists of multiple parts. Understanding each component's role is essential to obtaining a good separation and analysis. The carrier gas is required to provide a gas flow that transports the chemicals through the column and to the detector. The GC uses gas filters and flow regulators to supply high-purity, steady-flow gas. The purity and flow of the carrier gas determine good separation and consistent chromatograms.

In order to facilitate separation and detection, the carrier gas should preferably be inert. Helium, hydrogen, and nitrogen are a few of the often used carrier gases. The sample,

the type of detector, and the many attributes and traits of these gases all play a major role in the choice. When selecting carrier gases, one should take into account the necessary separation resolution and efficiency, as well as price and detector compatibility.

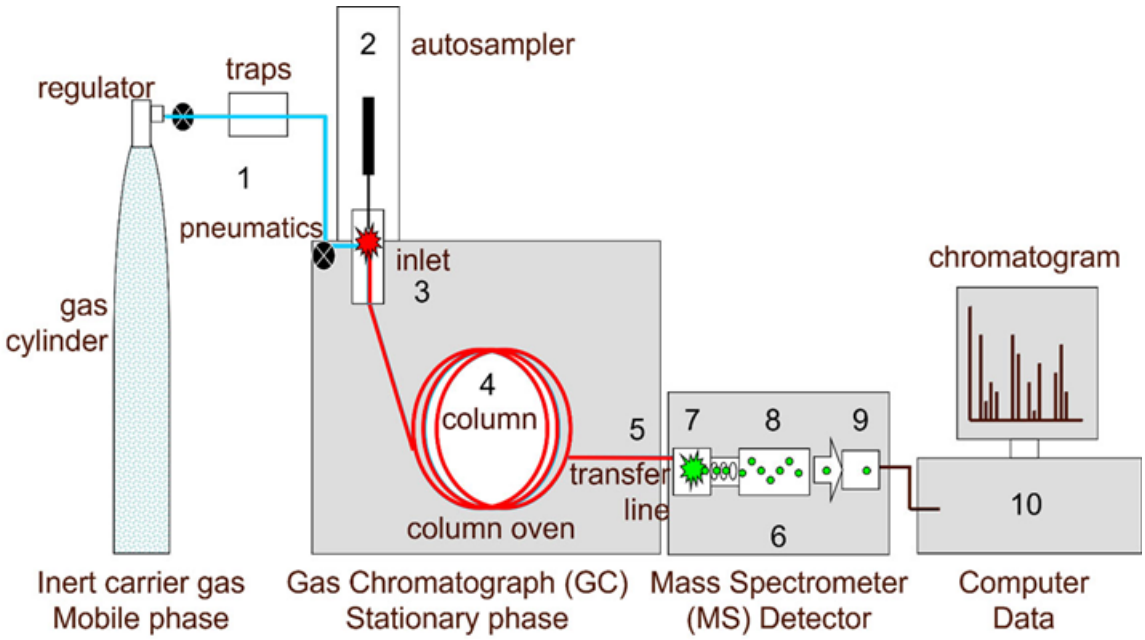

Fig. 20.1 Instrumentation of GC-MS

Working Procedure:

- Through the GC intake, the sample enters the carrier gas after being manually or mechanically introduced into the GC. If the sample is liquid, it will be evaporated by the heated GC intake before being sent to the analytical column as vapor.
- The components of the sample, known as “analytes”, are separated according to how differentially they partition between the carrier gas and the liquid stationary phase (which is kept inside the column); for more volatile gases, this is determined by their adsorption by a solid stationary phase. A liquid phase housed in a short (10-30 m length) and narrow (0.1-0.25 mm internal diameter) column is the most commonly utilized stationary phase in GC-MS analysis.
- Separating the neutral molecules and eluting them into the mass spectrometer via a heated transfer line is not required for GC-MS analysis unless the analytes are isomers.
- Inside the mass spectrometer, the neutral molecules are first ionized, usually via electron ionization (EI). In electrolysis (EI), a filament-produced electron is accelerated to 70 electron volts (eV), where it removes one electron from the molecule to produce a radical cation molecular ion. This high-energy ionization may result in an unstable molecular ion, and any excess energy may be released through

fragmentation. Both chemical rearrangements and bond breaks can result in the loss of a radical or neutral molecule. This all culminates in a number All of this leads to a number of, often a very large number of, ions of varying weights, with the molecule ion being the heaviest and fragment ions having varied lower masses, depending on:

1) The chemical composition
2) The molecular structure of the analyte
3) Where bond breakage has occurred
4) Which component still carries the charge

Using a mass analyzer based on their m/z, the ions of different masses are then separated in the following step. Mass resolution is the ability of the mass analyzer to distinguish between ions with incredibly small m/z changes. Unlike unit mass resolution instruments, which can only differentiate nominal masses to one decimal place, high mass resolution (HRMS) instruments may do so to four or five decimal places.

When the ions reach the ion detector, the mass analyzer separates them according to their m/z, and either an electron multiplier (for most low-resolution MS) or a multi-channel plate amplifies the signal. The acquisition software on each data point provides a mass spectrum and a chromatogram.

20.1.4 Applications of Gas Chromatography-Mass Spectrometry:

There are several uses for gas chromatography-mass spectrometry, such as identifying potentially hazardous materials in food, measuring organic contaminants in water, and examining petroleum compounds during the oil refining process. To identify unknown volatile chemicals, GC-MS is used in a variety of industries, including forensics, petrochemical, chemical, agricultural, tobacco, pharmaceutical, healthcare, energy, mining, and environmental research.

Food and Beverage Evaluation:

GC-MS analysis is necessary to ensure the safety and authenticity of the foods and beverages we eat. The important information that GC-MS systems provide on the safety of our food supply, such as the identification of pesticide residues and the description of ingredients, may be useful to manufacturers and regulatory agencies.

Analysis of the Environment:

GC-MS is a useful tool for tracking contaminants in soil, water, and air. It is particularly useful for measuring volatile organic chemicals (VOCs), semi-volatile organic compounds (SVOCs), brominated flame retardants, organochlorine pesticides, and polycyclic aromatic hydrocarbons (PAHs).

Metabolomics:

Metabolomics GC-MS offers some of the sophisticated analytical instruments required for thorough metabolomic investigations. It allows researchers to go deeper into the metabolome and obtain thorough coverage of metabolites, which in turn encourages research into primary and secondary metabolites in plants, animals, and microbes.

Analysis of Oil and Gas:

At different stages of the petroleum and natural gas testing procedures, GC-MS can be used to assess the energy content, CHA, SIMDIS, and H2S/organic sulfur content of natural gas and natural gas condensates. Furthermore, GC-MS analysis in refinery gas analysis (RGA) and detailed hydrocarbon analysis (DHA) can detect oxygenates, aromatics, BTEX compounds, and PAHs in crude oil.

Doping Test:

The main technique used in sports anti-doping labs to check athlete urine samples for illicit performance-enhancing drugs like anabolic steroids is GC-MS.

In Medicals:

Newborn screening tests, especially those that employ gas chromatography-mass spectrometry, can now detect inborn errors of metabolism (IEM), also referred to as congenital metabolic abnormalities. GC-MS can identify compounds even in urine with low quantities. Although they are typically missing, some chemicals do manifest in individuals with metabolic disorders. This approach to IEM diagnosis is growing in popularity and produces better results because it allows for early diagnosis and therapy initiation. It is now possible to screen a newborn for more than 100 genetic metabolic illnesses by doing a urine test based on GC-MS.

20.2 INTRODUCTION TO LC-MS:

The analytical technique known as liquid chromatography-mass spectrometry, or LC-MS, is extremely sensitive and specific. LC-MS stands for liquid chromatography combined with mass spectrometry. If additional components are present, they can be separated using Liquid Chromatography (LC), and the resulting eluent samples can subsequently be sent to Mass Spectrometry (MS) for identification, detection, and mass determination. Pharmaceutical medication components, intermediates, and related compounds are analyzed both quantitatively and qualitatively using LC-MS.

Liquid Chromatography Mass Spectrometry (LC-MS/MS) has emerged as a crucial tool for regular bioanalysis in many lab settings in recent years. This technique is now widely acknowledged and regarded as essential. Because of its remarkable sensitivity, LC Mass

Spectrometry is a very useful analytical method that is applied to both qualitative and quantitative analysis. It has shown to function exceptionally well and has been widely used in a variety of designs.

Chromatographic separation of analytes and subsequent mass-based detection are both components of the analytical process of LC mass spectrometry. Because of its excellent sensitivity, selectivity, and accuracy, liquid chromatography-tandem triple-quadrupole mass spectrometry (LC-MS/MS) has become widely used. Drugs, drug and food metabolites, biomarkers of disease progression or therapeutic efficacy, pesticides, food contaminants, indicators of ecosystem stability, and natural product extracts are among the analytes that this technique is most suited to detect at nanomolar or even picomolar concentrations.

A potent analytical method that combines two selective separation techniques is liquid chromatography-mass spectrometry (LC-MS). Even in extremely complicated combinations, this makes it possible to isolate, detect, and quantify the analytes of interest. By contrasting the measured analytes with established reference standards, LC-MS accomplishes this. Mass spectrometry (MS) is used to distinguish compounds according to their mass-to-charge ratios, whereas liquid chromatography (LC) is used to distinguish compounds according to their physico-chemical characteristics. By using its unique mass spectrum, the mass spectrometer can act as the "LC detector" and perhaps identify the species that correspond to each chromatographic peak. This lessens the need for chromatography to separate isotopic or mass-differentiated isobaric components. LC-Mass Spectrometry is a very useful analytical technique for detection, separation.

Nowadays, the most widely used bioanalytical method for quantification is the LC-MS/MS technique, usually referred to as liquid chromatography tandem triple-quadrupole mass spectrometry (MS/MS). Regarding sample preparation and chromatographic setup, the LC/MS/MS technique is comparable to HPLC/UV. Larger sample quantities can be analyzed with lower quantitation limits thanks to MS/MS's improved sensitivity and selectivity over UV detection. The use of LC/MS/MS is a useful, robust, and extremely responsive technique applied to a wide variety of low molecular weight substances. Furthermore, this specific technology can be automated and examined without the need for human oversight.

20.2.1 Principle of LC-MS:

The components of a mixture are separated using a High-Performance Liquid Chromatography (HPLC) system in the LC-MS technique. These components are then ionized and partitioned according to their mass-to-charge ratio. The separated ions are then sent in the direction of a detector that can identify and gauge the concentration of individual ions, such as an electron multiplier tube or photoelectric detector. In mass spectrometry analysis, the ion source is crucial since it makes it easier to produce ions efficiently for

analysis. To ionize intact molecules, a variety of ion sources are frequently used, such as APCI (Atmospheric Pressure Chemical Ionization) and ESI (Electrospray Ionization). The target analyte's chemical properties, particularly its polarity or non-polarity, determine which ion source should be used.

Since it allows for molecular-level analysis, this technology has several advantages, including increased sensitivity, specificity, and precision. Additionally, the analyte's structural properties can be clarified.

Mass Spectrometry Detection with Liquid Chromatography:

- It is not practical for the LC eluent containing the analytes to flow directly into the mass spectrometer, even though there are a number of detectors with varying sensitivities and technologies for analyzing a variety of sample types when combined with LC. The mass spectrometer (MS) has become a highly selective, sensitive, and universally applicable detector that provides enhanced selectivity, sensitivity, and efficiency. The interface, also known as the ion source, acts as the coupling mechanism between the LC MS system and the mass spectrometers. The LC is operated under normal back-pressure conditions.
    - The mass spectrometer's detector is used in a vacuum setting.
    - When the column eluent flows, heat and voltage are applied to the solvent, causing ionization or evaporation. The molecules of the analyte that have an electric charge are then added to the contact. Because the mass spectrometer can only detect and measure the ionized particles, this step is essential.
    - Atmospheric Pressure Ionization (API) is the process by which analyte ions are produced at atmospheric pressure within the interface; the interface is known as the API source. The two most common ionization sources used in liquid chromatography-mass spectrometry are electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI).
    - Both magnetic and electric fields are used to alter the ions of interest as they are drawn toward the mass spectrometer. Ion trajectories are transformed by changing the applied fields, which causes them to separate based on their mass-to-charge ratios respectively.
    - After separation, a variety of mass detectors can be used to detect and accumulate ions. A common primary mass detector is the electron multiplier. The unattached ions cause secondary electrons to be released when they collide with the electron multiplier's surface. On a dynode, this procedure takes place.
    - These secondary electrons are multiplied by transferring them via a sequence of dynodes.

The mass spectrometer measures and correlates the current amplification brought on by the secondary electron flow with the ion intensities it detects at a given time.

Rapid hardware changes are modulated and tracked by integrated software, and differentiation is made possible by reported mass-to-charge ratios that are based on the electromagnetic fluctuation in each quadrupole as ions approach the detector.

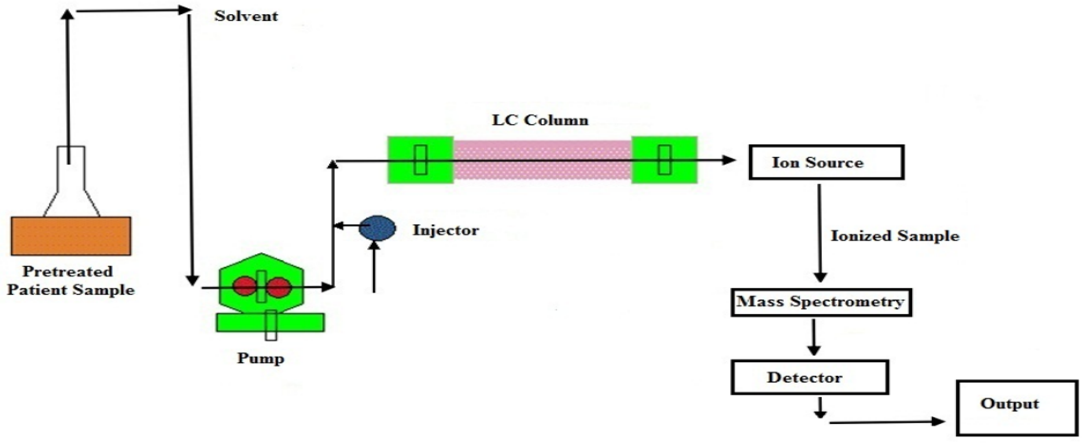

Fig.: 20.2: Schematic Representation of LC-MS

20.2.3 Instrumentation of LC-MS:

The Liquid Chromatography-Mass Spectrometry (LC-MS) technique is a hybrid of Liquid Chromatography and Mass Spectrometry that offers the separation capabilities of High-Performance Liquid Chromatography (HPLC) coupled with the detection capabilities of Mass Spectrometry (MS). Therefore, the two basic instrumentation components are:

- Liquid Chromatography
- Mass Spectrometry

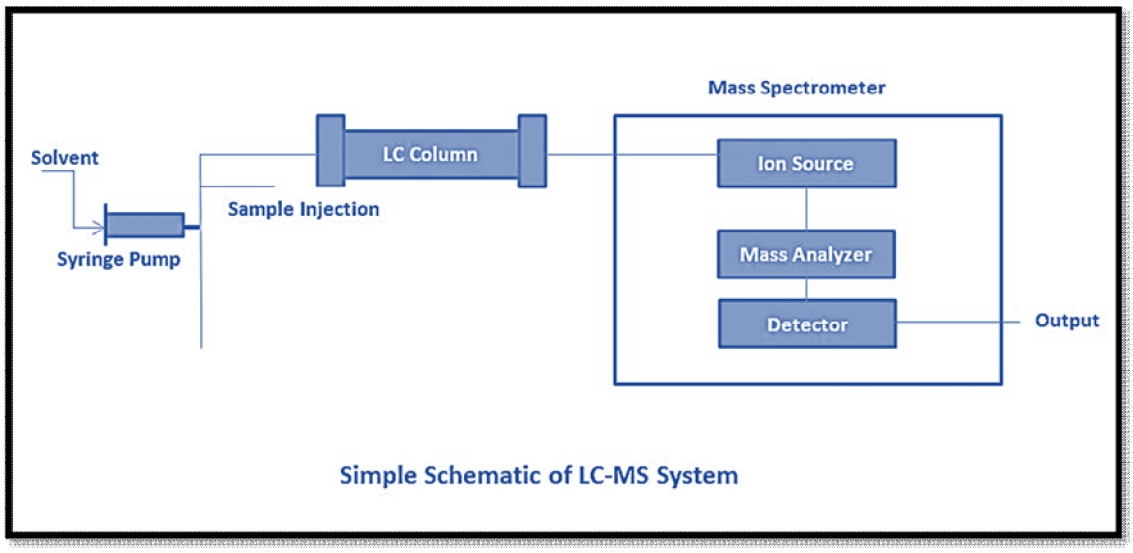

Fig. 20.3 Simple scheme of LC-MS

Liquid Chromatography:

Using a liquid mobile phase and a solid stationary phase, liquid chromatography (LC), a type of high-performance liquid chromatography, allows mixture components to be separated. There are several types of chromatography, such as affinity liquid chromatography, chiral separation, ion-exchange liquid chromatography, normal phase liquid chromatography, and reversed-phase chromatography. It is feasible to separate complex mixtures with small amounts by using a variety of highly effective column packing techniques. Here is a list of the elements that make up High Performance Liquid Chromatography (HPLC):

- **Pump:** The material is made up of components that are insensitive to solvents or any mixture of organic solvents and aqueous buffer. The device can supply a sizable amount of mobile phase, with a maximum flow rate of 10 milliliters per minute. Syringe pumps, constant pressure pumps, and reciprocating pumps are the three main types of pumps that are used.
- **Sample injector**: It provides a sample volume to the chromatographic device by being put into it. Samples are usually injected in volumes ranging from 1 to 100 iL. Up to 2 mL of injection volume is possible with injector loops. The two most popular kinds are manual and automatic injectors. Automatic injectors are more user-friendly, more accurate, and more comfortable for the patient than their manual counterparts.
- **Column**: It is a stationary phase composed of a carbon chain and silica material. Column lengths typically fall between 50 and 300 mm. HPLC columns use octadecyl (C18), octyl (C8), cyano, amino, and phenyl packings. Different kinds of columns are needed for different kinds of chemicals.
- **Detector and recorder**: The most important parts are the HPLC detectors. The signal from the detector can be recorded as a peak, and the associated information can be stored in a computer program.

Mass Spectrometry:

The analytical technique known as mass spectrometry depends on figuring out the mass-to-charge ratio of the ionic species connected to the analyte under investigation. A useful technique for figuring out an analyte's molecular mass and elemental makeup as well as for offering a thorough structural explanation of the analyte is mass spectrometry (MS).

Parts of the LC-MS System:

The interfaces and the ionization source are the two primary components of an LC-MS system. Below is a breakdown of the many components of a mass spectrometer:

1) Sources and Interfaces of Ionization
2) Analyzers of Mass

Ionization Sources and Interfaces:

Methanol, acetonitrile and water are the usual liquid components that are separated using the liquid chromatography process. The mass spectrometer's ion source is filled with the liquid combination of components. High vacuum conditions are used to run the ion source. Pressure differentials make it difficult to vaporize liquid drops while maintaining the component composition. Interfaces are used to solve this problem. Below is a list of the several interface types used in mass spectrometry.

Direct liquid Introduction (DLI):

Chemical ionization and solvent reagent gas vaporization are the methods used to achieve DLI ionization. Both normal and reverse phase solvent systems have been used. As reverse-phase solvents, methanol/water and acetonitrile/water (up to 60% water) are frequently used. Salt-containing buffers are generally not advised since they run the danger of capillary clogging when heated. DLI functions by combining the impacts of liquid flow rate and heat energy. There are restrictions on the liquid's flow rate as it enters the interface. A capillary intake or pinhole diaphragm is then used to transport the analyte ions produced by heat energy to the ion source.

Atmospheric-Pressure Ionization (API):

The three processes that make up atmospheric-pressure ionization (API) are nebulization, evaporation, and ionization. The two primary API techniques are atmospheric-pressure ionization (APCI) and electrospray ionization (ESI). Atmospheric pressure ionization (API) creates a mist of tiny droplets when a solvent containing a sample is nebulized in a large chamber after being delivered via a thin capillary tube. Some droplets have a positive or negative electric charge as a result of ionization. Large heating chambers are used to evaporate the solvent. As the solvent evaporates, the droplets get smaller. Molecules and ions collide. Through a capillary, ions entered the mass analyzer. A wide variety of moderate molecular weight analytes, both polar and non-polar, are subjected to the Atmospheric-Pressure Ionization (API) approach.

Electrospray Ionization (ESI):

The liquid sample is passed through a stainless steel capillary tube that is maintained at a high positive or negative electric potential of roughly 3-5 kV in order to perform electrospray ionization (ESI). At the capillary tip, charged droplets are created and then vaporized. Droplets get smaller and their surface charge rises as a result of the solvent

evaporating from them. When highly charged droplets transform into gas phase ions, the collision stops. The gas-phase ions enter the low-pressure area of the ion source after passing through the capillary sampling orifice. The primary advantage of ESI is the multiply-charged ions, which increase by 1 to 3 for molecules 1000 Da or more than 50000 Da. The m/z ratio is consistently less than 2000. The molecular weight is determined by ESI-LC-MS.

Atmospheric Pressure Chemical Ionization (APCI):

Analyte evaporation/desolvation and charged transfer processes in the vapor phase to generate vapor phase ions are the two steps in APCI. Using a tiny capillary tube, a solvent containing a liquid sample is nebulized into a huge chamber as part of the Atmospheric Pressure Chemical Ionization (APCI) process. Small droplets are created as a result of solvent evaporation, which takes place in a large heating chamber at atmospheric pressure. There is ionization. The usual temperature range for ionization is 250–400 °C. Through chemical reactions, the ions give molecules charges. The mass analyzer's capillary aperture allows the ions to pass through. This method is frequently used for analytes of moderate molecular weight that are either non-polar or have low polarity.

Thermo spray and Plasma spray Ionization (TSPI):

By heating a capillary tube that a liquid sample solution is passed through, thermospraying causes the solvent to evaporate. Droplets of charge are produced. Solvent evaporation causes the droplets to shrink in size. The droplets' surface charge density rises. After that, the ions are put into a mass analyzer that has an electrostatic voltage system installed. Ions can be enhanced in thermal spray by plasma or corona discharge, however plasma spray does not create ions. Neutral compounds are more ionized by electric discharge. improved ionization of molecules. Plasma spray is used in clinical and medical analysis because to its higher sensitivity.

Atmospheric pressure photo Ionization (APPI):

APPI excites and ionizes molecules using photons. The two main steps of APPI are ionizing and exciting the analyte from the eluent. Like APCI, APPI uses LC eluent vaporization. APPI produces photons using a Kr lamp. Kr lamp emits high-energy photons that excite and ionize molecules. Analyte ionization is reduced by energy range. Analytes that have been ionized enter a mass analyzer. Analytes with non-polar properties that are difficult to ionize using Electrospray Ionization (ESI) and Atmospheric Pressure Chemical Ionization (APCI) procedures benefit from this approach.

Particle Beam Ionization:

A particle beam interface developed by Browner et al. allows solvents and solutes to be separated with little loss of solute. There are similarities between the nebulization and

evaporation processes and electrospray ionization (ESI), atmospheric pressure chemical ionization (APCI), and thermo spray (TSP). For separation, the eluent from LC or HPLC is run through a thin tube. Injection of helium gas produces a spray of high-velocity liquid droplets. The liquid droplets in the nebulizer shrink in size as a result of evaporation in the heating chamber. As a beam of particles, the liquid droplets leave the heating chamber. Similar to Atmospheric Pressure Chemical Ionization (APCI) and Electro Spray Ionization (ESI), the beam then passes through an ionization chamber.

Continuous Flow Fast Atom Bombardment (FAB):

The FAB is a straightforward and extremely sensitive interface detection method. The target is bombarded with fast atoms, like xenon or argon (Ar), in FAB liquid. Glycerol is used to dissolve the sample before applying it to a thin metal plate or probe. The probe is placed inside the mass spectrometer, where a beam of rapidly moving atoms bombards the samples, ionizing them. The mass-to-charge ratio (m/z) of the resultant ions is then used for analysis. Bulky and thermally labile compounds are analyzed using FAB. It is applied to proteins and surfactants.

Mass Analyzer:

The ions are sent to a mass analyzer after ionization, where their m/z ratio is used to separate them. Usually, mass analyzers are assessed according to their responsiveness, rate, speed, and time. Below is a discussion of the mass analyzer's main parts:

Quadrupole Mass Analyzer:

It is currently the most widely used and useful mass analyzer on the market. Between an ion source and a detector are two rows of parallel rods. The mass analyzer, which uses an ion's m/z to separate it in space or time. Four cylindrical or hyperbolic rods arranged in a radial array make up the linear quadrupole mass analyzer. A DC potential and an oscillating radio frequency voltage are applied to opposite rods. Ions of one m/z are stabilized by applying DC and RF to rods, and these ions are then sent to the detector. Unstable m/z ions are released by the rods. Ions are introduced to the quadrupole using a low-accelerating potential. Ions oscillate perpendicular to the length of the rod as they pass through the quadruple filter. m/z-carrying ions are driven toward the detector by applying DC and RF voltage in a fixed ratio. DC/RF proportionDC/RF ratio determines resolution. The Quadrupole typically scans at 1000 m/z at <4000 m/z. Mass accuracy rarely exceeds 0.1 m/z due to unit mass resolution. RF values are usually 1-2MHZ. 1000V DC and 6000V RF are possible.

Time of Flight Analyzer:

A flexible technique for a range of ion sources and intake systems is time of flight. The system just needs simple electrostatic calibration and maintenance because it doesn't

have a magnetic field. A voltage accelerates and extracts the ions. The mass and charge of the ion determine how long it takes to move along the drift or flight route. For solitary charged ions (z=1, m/z = w), the period of flight is exactly proportional to their mass. As they approach the detector, lighter ions will arrive before heavier ones. Scanning allows for the simultaneous detection of every ion. For high m/z values, the mass range scanning is quick and appropriate.

Ion Trap Mass Analyzer:

High resolution, sensitivity, and the capacity to conduct repeated product ion scans are all demonstrated by the Ion Trap Mass Analyzer. A three-dimensional ion trap is the quadrupole ion trap. A cylindrical ring electrode exposed to a quadrupole field makes up the device. End-capped electrodes are two more kinds of electrodes. While the opposite electrode of the end cap has several apertures or holes for the passage of ions to a detector, the first electrode has a single central aperture for the entry of electrons or ions into the trap. The presence of helium bath gas in the trap stabilizes the ion trajectories. The collision takes place between ions and a bath gas of helium. Ion motion improves the analyzer's trapping effectiveness. The mass spectrum is generated by selectively releasing ions from the trap based on their mass-to-charge (m/z) ratios.

Fourier Transfer Ion Cyclotron Resonance (FT-ICR):

One very important mass analyzer is the Fourier transform ion cyclotron resonance (FT-ICR). Following their introduction into the mass analyzer, the ions produced by the ionization source are separated according to their individual mass-to-charge ratios (m/z). Circular trajectories confine the ions that are introduced into the chamber. The combined effect of magnetic and electric fields accelerates ions. This phenomenon causes the ions to become excited, which results in a current that fluctuates over time. While trapped, the ions are separated according to their mass-to-charge (m/z) ratios.

Detectors:

Because it produces an electrical signal that is exactly proportionate to the number of ions that impact it, the detector is an essential part of the mass spectrometer. Following their passage through the analyzer, the ions must be found and transformed into a signal. The list of frequently used detectors is as follows.

Point Ion Collectors Detector:

The mass spectrometer's ion collectors are positioned at particular locations. Every ion is focused on a single detection spot. Ion detection can be recorded by measuring electric current and then recorded for further examination. The amount of ion influx observed at a particular location on the ion detector is directly proportional to the electric current magnitude.

Array detector:

A flat configuration of point collectors is called an array detector. An array detector picks up the ions at a certain spot or across a plane. A planar ion collector is used to detect the ions after they have been separated according to their mass-to-charge ratio (m/z). Ions with various masses can be simultaneously and spatially discriminated detected using an array detector.

LC-MS Analysis:

The extensive use of LC-MS for analysis has helped both large proteins and small compounds in a range of matrices. The detection of alkylphenol ethoxylates (APE), the measurement of genotoxic contaminants in active pharmaceutical ingredients, the identification of twelve model compounds in exhaled breath that correspond to particular classes of doping agents in athletes (such as stimulants and anabolic agents), the identification of contaminants in food items and dietary supplements, and the measurement of drug metabolites in biological samples are some examples of practical uses for this technology.

20.2.3 Application of LC-MS:

LC-MS is a widely used analytical technique in sample analysis that combines liquid chromatography (LC) and mass spectrometry (MS). Modern mass spectrometry's great sensitivity has made it easier for liquid chromatography-mass spectrometry (LC-MS) to replace several immunoassays. Because of its remarkable sensitivity and specificity, LC-MS has been used to improve efficacy in the drug discovery process. Accurate and reliable measurements are made possible by the method's integration with stable isotope dilution.

Biomedical Applications:

The use of the LC-MS technology is beneficial for both generating a thorough analysis of endogenous steroids and identifying steroid medications found in bodily fluids. For the preliminary analysis of amino acids, LC-MS in conjunction with laser desorption and thermospray was utilized. Using LC-MS in conjunction with electrospray, the molecular weights of proteins, peptides, saccharides, nucleosides, and nucleotides were determined. Thermospray ionization and liquid chromatography-mass spectrometry have been used to evaluate bile acids.

Environmental Applications:

The analysis of a variety of materials, such as soil, drinking water, wastewater, air, and sludge, is frequently conducted using LC-MS. The specimens in question may represent a wide range of chemical entities, from ionic organometallic moieties to apolar hydrocarbons. Numerous pesticides and herbicides, including sulfonylurea herbicides, triazine derivatives, chlorophenols, and phenoxyalkanoic acids, can be analyzed using LC-MS.

Biochemical Screening:

LC-MS analysis of blood samples is used to identify metabolic abnormalities in infants. Second-tier liquid chromatography-mass spectrometry (LC-MS) analysis has been used in neonatal screening to validate the results of initial immunoassays.

Pharmaceuticals:

LC-MS is frequently used to identify therapeutic compounds, with chiral medicines being isolated with special attention. Thermospray has been used to study potential antimalarial drugs and antibiotics. There have been reports of the effective use of LC-MS in the detection of bromazepam and similar compounds in intoxication cases. One important field of research is the use of LC-MS in the identification, separation, and purification of drug metabolites. This is because drug metabolites are frequently thermally or chemically labile, which makes liquid chromatography necessary.

Vitamins and Related Metabolites:

When it comes to measuring vitamin D and its metabolites, LC-MS has been a popular method. Assays for the identification and measurement of 25-hydroxyvitamin D2 and D3 in plasma and serum samples have been developed using liquid chromatography-mass spectrometry (LC-MS). Similar assays are available for the fat-soluble vitamins, specifically vitamin K15 and vitamin E13,15.

Steroid Hormones:

In the field of steroid biochemistry research, the use of LC-MS analysis has been beneficial, especially when traditional immunoassays have shown limited efficiency. To measure low levels of testosterone and dihydrotestosterone in females and children, sensitive LC-MS assays have been developed.

20.3 SUMMARY:

These days, LC-MS/MS, or liquid chromatography (LC) tandem triple-quadrupole mass spectrometry (MS/MS), is the most popular bioanalytical technique for quantification. In terms of chromatographic setup and sample preparation, the LC/MS/MS process is comparable to HPLC/UV. However, MS/MS provides better sensitivity and selectivity than UV detection, enabling higher throughput tests with lower quantitation limits that are more sensitive. For a wide range of tiny compounds, LC/MS/MS is a useful, reliable, and sensitive technique. Additionally, this technique may be automated and analyzed without human intervention.

20.4 TECHNICAL TERMS:

Gas Chromatography, Liquid Chromatography, Assays, Immunity, etc.,

20.5 SELF-ASSESSMENT QUESTIONS:

1) Write about GC-MS
2) Write about LC-MS
3) Give applications of GC-MS and LC-MS

20.6 SUGGESTED READINGS:

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