UNIT 1 DEFINITION AND IMPORTANCE OF QUALITY

Structure

1.0 Objectives
1.1 Introduction
1.2 Definition of Food Quality
1.3 Food Quality Attributes
1.4 Quality Specifications for the Consumer
1.5 Food Borne Hazards/Food Poisoning
Microbial Infections
Water and Food Associated Viruses
Preventive Measures
1.6 Functions of Quality Control
1.7 Let Us Sum Up
1.8 Key Words
1.9 Answers to Check Your Progress Exercises
1.10 Some Useful Books

1.0 OBJECTIVES

After reading this unit, you should be able to:

- define food quality;
- state the quality specifications for consumers;
- classify and explain food quality attributes; and
- explain food borne hazards and infections.

1.1 INTRODUCTION

Food quality and safety are the two of the most important aspects of food processing. Therefore an understanding of what constitute food quality is very essential. Equal or more important is ensuring food safety. Food poisoning through microbial and viral means is a major concern.

An efficient quality control department in a food processing unit plays vital role in ensuring both quality and safety. These aspects are dealt with in this unit.

1.2 DEFINITION OF FOOD QUALITY

Quality of foods may be defined as the composite of those characteristics that differentiate individual units of product; these characteristics should have significance in determining the degree of acceptability of that unit by the buyer.

Some important characteristics of the food / food products are:

- Colour and gloss, viscosity and consistency, size and shape, texture and flavour.
- Nutritive values (vitamins, minerals) – hidden attributes.

Quality is commonly thought of as degree of excellence. It may be considered as a set of specifications, which are to be met within given tolerances or limits. Quality Control may be defined as the maintenance of quality at levels and tolerances acceptable to the buyer while minimizing cost for the vendor.

1.3 FOOD QUALITY ATTRIBUTES

The quality attributes referred to as sensory may readily be classified in accordance with the human senses by which they are perceived. The sensory attributes namely, the senses of sight, touch, taste and smell are given in Table 1.1.

Table 1.1: Classification of quality attributes

Sight	—	• Appearance • Colour • Gloss • Viscosity • Size and shape • Defects
Touch	—	• Kinesthetic (texture) • Hand or finger feel • Mouth feel
Smell and taste	—	• Flavour • Odour • Taste
Hidden	—	• Nutritive value • Adulterants • Toxicity

Some of the important sensory quality attributes are described below:

Colour and gloss

Colour is an appearance property due to spectral distribution of light. Glossiness, transparency, haziness, and turbidity are properties of materials due to the differences in reflectance and transmittance of light.

Physically, colour is a characteristic of light, measurable in terms of intensity and wavelength. It arises from the presence of light in greater intensities at some wavelengths than of others.

Light may be reflected, transmitted, absorbed, or refracted by the object being illuminated. Spectrophotometers are used to measure colour of the products.

Viscosity

Viscosity or consistency is an appearance property of great importance to food products such as ketchup, creams, juices, pulp, jams, jellies, syrups, etc. The measurement may be used to indicate the consistency of the products. It may also be used as an index to the amount of ingredient in the product.

Liquids flow as if they were composed of individual layers. The friction resulting from the resistance to flow between the liquid layers is called apparent viscosity. Brooke field or Ostwald viscometer is used to determine the viscosity of the product.

Size and shape

Grading into various size and shape categories is usually one of the first steps in food processing operations. This may be accomplished by hand or by means of mechanical sorters, using screens, reels, slots, etc. Grading helps in maintaining uniformity.

- *Defects*

  Defects have been defined as "imperfections, due to the absence of something necessary for perfection, or the presence of something that distracts from perfection".

  In grading foods for defects, tolerances may be established in terms of maximum numbers of defective units allowable.

  Defects may be classified into:

  1. Genetic – physiological,
  2. Entomological,
  3. Pathological,
  4. Mechanical, and
  5. Extraneous or foreign matter.

Kinesthetics of texture

Kinesthetic characteristics deal with the sense of feel. They can be classified as follows:

Finger feel:

- Firmness as encountered by the customer selecting a firm apple, measured physically by compression.
- Softness or yield quality as in selecting a peach, or plum measured physically by compression.
- Juiciness as in immature sweet corns where the thumbnail is used to test the ease and amount of juice squeezed out of a kernel.

Mouth feel:

- Chewiness, as sensed by the resistance of the product to compression and shearing action of the teeth.
- Fibrousness as sensed by the presence of an inedible residue remaining in the mouth after mastication, as well as resistance to cutting forces of the teeth.
- Grittiness, as sensed by the presence of small grit particles, such as sand, or stone cells.
- Mealiness, as sensed by the coating of starch or other material with adhesive properties, over mouth tissues.

- Stickiness, as sensed by the mouth while chewing foods with adhesive properties.
- Oiliness, as sensed in the mouth, caused by oily or soapy products.

The characteristics may be determined by tenderometers, texture meters, puncture meters, succulo meters, fibro meters and pressure testers.

Flavour

Flavour includes taste and odour/ aroma. Taste is a four-dimensional phenomenon, consisting of sweet, sour, salt and bitter. Sweetness can be measured by use of refractometers, sourness by pH meters, and saltiness by flame photometer or by argentometric titration. Bitterness is not estimated but compared to that of quinine sulfate. Odour/ aroma is determined by gas chromatography.

Hidden characteristics

The hidden characteristics of quality are those, which the consumer cannot evaluate with his senses, and yet are of real importance to his health and economic welfare. Nutritive value is one of the hidden characteristics, which is now considered by the consumer as an important attribute. Adulterants and toxins are the other hidden characteristics. Toxins can be microbial toxins, pesticide residues or heavy metals.

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Define food quality.

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2. Explain the important quality characteristics of food.

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3. What constitute the hidden characteristics of food?

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1.4 QUALITY SPECIFICATIONS FOR THE CONSUMER

The Quality Control System – should be properly geared to meet the consumer's specifications, the quality control cycle usually begins and ends with the customer's specifications.

1st Step : Determine what are the customer's specifications for the product.

2nd Step : Set up procedures to measure these specifications, objectives; scientific methods should be used as far as possible.

3rd Step : Workout the sampling schedule and set up control points in the plant.

4th Step : Final inspection of the finished product.

1.5 FOOD BORNE HAZARDS / FOOD POISONING

In recent years, a number of bacteria, viruses and parasites have emerged as food-borne pathogens resulting in numerous food-borne disease outbreaks. Genetic changes in microorganisms resulting in increased virulence, changes in social attitudes and eating habits, changes in food production and distribution systems and demographic shifts are some of the factors responsible for this.

1.5.1 Microbial Infections

Bacillus cereus

Bacillus cereus is a Gram-positive, motile, spore -forming, facultative anaerobic rod. It is ubiquitous in nature, commonly found in soil (especially rice, paddy, soil) and vegetation. It has been isolated from many foods, including cereal and cereal derivatives, spices, milk and dairy products, vanilla sauce, recipe dishes, chicken soup, mashed potatoes, vegetables, rice dishes and dried foods.

Bacillus cereus can cause two distinctive forms of food poisoning caused by enterotoxins. Emetic food poisoning is caused by the ingestion of emetic toxin that has been pre-formed in food. It causes general malaise, nausea and vomiting and occasionally diarrhoea. This type of food poisoning has been linked with starchy foods such as cooked rice, pasta and noodles. Diarrhoeal

food poisoning is caused from the formation and release of enterotoxin in the small intestine. However, the enterotoxin can also be pre-formed in food.

Clostridium botulinum

Clostridium botulinum is a Gram- positive rod. It is a spore-former and an obligate anaerobe. It is ubiquitous, so is widely distributed in soil and marine sediments throughout the world. It is also found in the intestinal tract of animals, including fish.

Non-proteolytic strains of Clostridium botulinum are psychrotrophic. It is because of their non-proteolytic characteristics, that their growth in foods cannot be detected by off-odours and off-flavours. The risk of toxin production prior to the food becoming unacceptable to the consumer is considerably higher than in those foods contaminated with proteolytic strains. In the spore form, it is resistant to heat treatments such as pasteurization. A heat process called a "Botulinum Cook" at high temperatures is commonly recommended for low acid canned products.

Clostridium botulinum produces preformed toxins. The toxin itself can be destroyed by heat treatment (80°C or above) for only a few seconds. Botulism is extremely serious and unless recognized and treated promptly, carries a high risk of mortality. It is the most severe form of food poisoning.

Most of the outbreaks of botulism have been associated with products of fish or marine animals, meat and fruit and vegetables - including mushrooms. Insufficiently heated, canned and bottled foods are at high risk as these provide the anaerobic environment required by the organism to grow. Other inadequately processed products such as farm-cured pork products or those produced where process control is insufficient e.g. traditional fermented products have also been implicated in outbreaks.

Salmonella

Salmonella is a Gram-negative motile rod. It is non-spore forming and facultatively anaerobic in nature. Pasteurization and equivalent heat treatments will generally destroy the organism.

The main source of *Salmonella* for man is food from infected food animals. These animals become infected via the environment, contaminated feed or water, or from other infected animals, birds or rodents. Therefore, meat, poultry, raw milk and eggs should be considered as potentially contaminated with *Salmonella.*

Types of foods involved in food borne salmonellosis have been wide -ranging, but involve mainly poultry and meat products, egg and egg products, cereal and grain products, desiccated coconut, chocolate and dairy products.

The clinical disease of *Salmonella* infection is gastroenteritis. It is one of the main causes of food borne illness the world over. Although death from salmonellosis is rare, it can occur in "at risk" groups, e.g. infants, the elderly and the immuno -compromised (such as hospital patients).

Listeria monocytogenes

Listeria monocytogenes is a Gram -positive, non-sporing rod. It is aerobic and facultatively anaerobic in nature. *Listeria monocytogenes* is psychrotrophic in

nature Of all the non-sporing, vegetative food pathogens, Listeria is the most heat resistant. It is, however, generally agreed that milk pasteurization will destroy normal levels of *L. monocytogenes* in milk.

Listeria is ubiquitous in the environment and so can be transferred to foods from a wide variety of sources. Infection from Listeria can also originate from direct or indirect contact with animals (sheep and cows can both excrete *L. monocytogenes* in faeces and sometimes in milk).

Listeriosis is a comparatively rare disease; however, because of the potential severity of the disease, measures for its control in foods are very important. It is generally agreed that the majority of cases of listeriosis are food borne and may be preventable. Symptoms are typically meningitis or septicaemia and in pregnant women it can cause a flu-like illness, which can result in miscarriage, stillbirth or birth of a severely ill infant.

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive coccus. It is a non-motile, non-sporing, facultative anaerobe. Staphylococcus aureus can grow within the temperature range 7°C – 48°C, with an optimum of 35°C – 37°C.

Although *Staphylococcus aureus* is a ubiquitous organism, the largest reservoir of enterotoxin producing staphylococci is man. Therefore, the presence of *staphylococci* in cooked or processed foods can serve to indicate poor hygiene amongst food handlers. Animals may also act as a source of *Staphylococcus aureus*. Typically raw milk and raw meat (particularly pork) may be contaminated with the organism. Some strains of *Staphylococcus aureus* are capable of producing heat-stable toxins (enterotoxins) in food. It is the toxin that causes the typical symptoms associated with *Staphylococcus aureus* food poisoning. Typical symptoms are nausea and vomiting with occasional abdominal cramping and diarrhoea. Foods involved in *Staphylococcus aureus* food poisoning are typically those that have been handled and then temperature abused prior to consumption. Foods implicated in Staphylococcus aureus food poisoning have been cooked meats (notably salted meat such as ham), poultry products, custard or cream -filled pastries, egg foods, cheese, prawns and salads containing potato.

Shigella spp

The genus Shigella consists of four species: *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C), and *S. sonnei* (subgroup D). -sporulating, non -motile rods in the family *Enterobacteriaceae.*

Shigellosis, although commonly regarded as waterborne, is also a food borne disease restricted primarily to higher primates, including humans. Food handlers with poor personal hygiene usually spread it among humans. Foods most often incriminated in the transmission have been potato salad, shellfish, raw vegetables, and Mexican dishes.

Vibrio cholerae

The genus *Vibrio* includes Gram-negative, oxidase-positive (except two species), rod- or curved rod-shaped facultative anaerobes. Many *Vibrio* spp. are pathogenic to humans and have been implicated in food borne disease. Pathogenic *V. cholerae* produces a heat-sensitive enterotoxin that causes the characteristic cholera symptoms, including "rice water stool."

1.5.2 Water and Food Associated Viruses

Several viruses like *Hepatitis* A, *Norwalk* and *Norwalk* like viruses; *Poliovirus* and *Echovirus* may cause food borne disease. Some of the other viruses that have also been associated with food are: *Astrovirus, Calcivius, Enteric Adenovirus, Parvovirus* and *Rotavirus*. These enteric viruses replicate in the intestine of infected individuals and are transmitted by faecal-oral route. The most common types of food-borne viral diseases are *Hepatitis A* (infectious hepatitis) and acute viral gastroenteritis.

The *Hepatitis* A virus (HAV) is one of more than 70 members of enterovirus group of *Picronaviridae* family. Food borne viral gastroenteritis is usually a mild disease with various degrees of nausea, diarrhoea, malaise, abdominal pain, muscle pain, anorexia, headache, and low-grade fever. Illness develops 20 to 50 hours after the consumption of contaminated food and lasts for 1 to 8 days.

Ice, water, ice cream, milk, pastries, salads, sandwiches, shellfish, and other foods consumed raw or subjected to additional handling after cooking are major food vehicles for virus transmission.

1.5.3 Preventive Measures

Prevention and control of food borne disease depends on careful food production, handling of raw products and preparation of finished products. Hazards can be introduced at any point from field to table. The 20th century witnessed revolution in food sanitation and hygiene including refrigeration, chlorination of drinking water, pasteurization of milk, potassium permanganate washing of root vegetables like carrots, lettuce etc. which was a consequence of applied technologies.

Chlorination of drinking water sources for food animals, sanitary slaughter and processing of meat, poultry and seafood, irradiation and other microbial reduction measures for raw agricultural commodities are significant as approaches for food safety. Hazard Analysis Critical Control Point (HACCP) process occurs when monitoring and control technologies are systematically applied to food production to prevent food borne illnesses.

Food cooks are the last but one point of critical control before meal is consumed, the last being those responsible for meal service on the table or in the dishes. Therefore, interventions to promote safe food practices are needed.

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Give three examples of microorganisms causing food poisoning and indicate the probable causes for the infections.

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2. Briefly discuss water and food borne viral infections.

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3. Suggest a few measures to prevent food poisoning.

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1.6 FUNCTIONS OF QUALITY CONTROL

A well functioning quality control organization will contribute to the reduction of rejects, maintenance of uniform quality, increased customer satisfaction and employee morale.

Some of the specific responsibilities of Quality Control Department are listed below:

- Inspection of supplies and materials.
- Scheduling of operations.
- Measurement of production efficiency.
- Inspection of the finished products.
- Shipping and storage controls.
- Preparation of specifications and procedures.
- Sanitation inspections.
- Conformance to food regulations.
- Waste disposal control.

The quality control department to be effective should have good liaison with the other departments like Sales and Purchase, Production, Research and

Development etc. and also should have complete support from the management.

Check Your Progress Exercise 3

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Indicate five functions of a quality control department.

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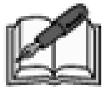

1.7 LET US SUM UP

Quality is the composite of those characteristics of which determine the degree of its acceptability by the consumer. The characteristics of food include measurable characteristics like colour and appearance, texture, flavour and also hidden characteristics like nutritive value, presence or absence of adulterants or toxicants.

There is a number of food borne infections. A large number of microorganisms are implicated in food poisoning. Besides, some water borne viruses are also of great concern.

An efficient quality control system in a food processing unit has a major role to play in maintaining consistent quality of finished products and ensure food safety.

1.8 KEY WORDS

Food quality : The composite of those characteristics of food determining the degree of acceptability by the consumer.

Colour : Colour is an appearance property due to spectral distribution of light.

Viscosity : The resistance to flow of a liquid is termed viscosity.

Kinesthetic of texture : Texture perception by feel.

Flavour : Combined sensory perceptions of taste and aroma.

Hidden characteristics	**:**	Nutrients content and presence or absence of adulterants and toxicants.
Botulism	**:**	Food poisoning caused by *Clostridium botulinum*.

1.9 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following:
   - Characteristics differentiating individual units.
   - Degree of acceptability
2. Your answer should include the following:
   - Colour, texture, appearance.
   - Nutrients, toxicants
3. Your answer should include the following
   - Nutrients, toxicants.

Check Your Progress Exercise 2

1. Your answer should include the following:
   - Clostridium botulimum, Salmonella, Staphylococcus aureus.
   - Insufficient heat processing of canned foods.
   - Poor hygienic handling of foods.
2. Your answer should include the following:
   - Hepatitis A, Norwalk, Astrovirus, Rotavirus.
   - Unhygenic handling of food after cooking.
3. Your answer should include the following:
   - Hygienic handling, chlorination, pasteurisation.

Check Your Progress Exercise 3

1. Your answer should include the following:
   - Inspection of raw materials.
   - Inspection of finished products.
   - Preparation of specifications and procedures.
   - Conformances to food regulations.
   - Waste disposal control.

1.10 SOME USEFUL BOOKS

1. Bibek, Ray (2001) Fundamentals of Food Microbiology, CRC Press, London & Washington DC.

2. Multon, J.S. (1995) Quality control for Foods and Agricultural Products, VCH Publishers, New York.

UNIT 2 QUALITY STANDARDIZATION

Structure

2.0 Objectives
2.1 Introduction
2.2 National Food Control Systems
2.3 National Food Legislations
PFA Act, 1954
Fruits Products Order, 1955
Vegetable Oil Products (Regulation) Order, 1998
Solvent Extracted Oil, De-Oiled Meal & Edible Flour (Control) Order, 1967
Meat Product Control Order, 1973 (Ministry of Rural Areas and Employment)
Edible Oil Packaging (Development and Regulation) Order, 1998
Milk and Milk Product Order, 1992 (Ministry of Agriculture)
Standard of Weights and Measures Act, 1976
Export (Quality Control and Inspection) Act, 1963
Bureau of Indian Standards Act, 1986
Agmark Grading and Marketing Act and Rules, 1937
2.4 Food Regulations for International Organizations
Codex Alimentarius Commission (CAC)
International Organization for Standardisation (ISO)
2.5 Let Us Sum Up
2.6 Key Words
2.7 Answers to Check Your Progress Exercises
2.8 Some Useful Books

2.0 OBJECTIVES

After reading this unit, you should be able to:

- state the importance of food control;
- describe various national food legislations; and
- international food regulations.

2.1 INTRODUCTION

Effective national food control systems are essential to protect health and safety of the domestic consumers. They are also critical in enabling countries to assure the safety and quality of their foods entering international trade.

Especially to facilitate international trade in food and food products, international agencies have laid down regulations. The national food regulations of most countries are being harmonized with these international regulations.

2.2 NATIONAL FOOD CONTROL SYSTEMS

Some of the **objectives** of the National Food Control Systems are:

- Protecting public health by reducing the risk of food borne illness.
- Protecting consumers from unsanitary, unwholesome, mislabelled or adulterated food.

- Contributing to economic development by maintaining consumer confidence in the food system.
- Providing sound regulatory foundation for domestic and international trade in food.

The **scope** of Food Control Systems should cover all food produced, processed and marketed within the country, including imported food. Such systems should have a statutory basis and be mandatory in nature.

2.3 NATIONAL FOOD LEGISLATIONS

The laws regulating the safety and quality of food in India date back to 1899. Before 1954, states or provinces in India had their own food laws. The variations in food standards created conflicts in inter-state trade.

2.3.1 PFA Act, 1954

In 1943, Central Advisory Board was appointed which recommended for Central Legislation. Consequently in 1954, Central Legislation called "Prevention of Food Adulteration Act (PFA Act)" was enacted in the parliament, which came into effect from 1st June 1955.

Ministry of Health and Family Welfare is responsible for ensuring safe food to the consumers. The objective of the Act is to ensure supply of pure and wholesome food to the consumers and also to prevent fraud or deception. Major amendments in 1964, 1976 and 1986 have been effective in making the punishments more severe and also empowering consumers and voluntary organizations to play effective role in food safety.

Some of the salient features of the legislation include:

a) Definition of term – adulteration.

b) Powers to consumer organizations to draw legal samples and initiate legal proceedings.

c) Regulation on quality of imported foodstuff.

d) Provision of a warranty by a manufacturer, distributor or dealer to vendor.

e) Provisions for seizure and disposal of perishable foods, unfit for human consumption by local authority.

f) Appointment of an Advisory Committee called Central Committee for Food Standards (CCFS) and its sub-committees.

Adulteration

Under the PFA the food is said to be adulterated:

a) If it does not meet the specifications prescribed in Appendix - B of PFA,

b) If it contains injurious substances,

c) If any inferior or cheaper substance has been added,

d) If any constituent is abstracted from the food,

e) If article had been prepared, packed or kept under unsanitary conditions whereby it became contaminated,

f) If an article of food consists wholly or in part of any filthy, putrid, rotten, decomposed or diseased substance or otherwise is unfit for human consumption.

The PFA Rules, 1955, are divided into XVII parts. Some of the parts, which are of direct consequence to the food preservation and processing industry, are discussed.

Part VI - deals the colouring matters. It lays down the list of permitted, natural and artificial colours, as well as the maximum limit of synthetic dyes which can be used and the list of food products where the colours can be used (Rule 23 to 31).

Part VII – deals with the packing and labelling of food. It lays down the labelling provisions, the details of the label, procedure for claiming nutritional status of the product and certain restrictions on the misleading statements. It also lays down the form of labels which is to be declared on different food products. The important rule under this part is Rule 37 A which deals with the proprietary food which is the major commodity available in the market now-a-days. The important provisions under this part have been incorporated recently for declaring "Best Before Date", Non-Vegetarian & Vegetarian, Irradiation and also prohibiting misleading claims. (Rule 32 to 43).

Part VIII – provides for prohibition and regulations of sales. It prohibits the sale of certain admixtures and various products regarding their labelling and other things. It also lays down the certification of various food colours as well as food additives under ISI Certification Mark Scheme of Bureau of Indian Standards (Rule 44 to 48 C).

Part IX – deals with the condition for sale and licence. Under this Part, condition for sale and condition of licence, duration- of licence and procedure for issue of licence have been provided. Manufacturing premises, manufacturing products, Requirements for Fruit & Vegetable Products, Meat and Meat Products, Vanaspati, Solvent Extracted Edible Oils (Rule 49 to 51).

Part X – deals with the preservatives. It classifies the preservatives, lays down the quantity of various preservatives which can be used and certain restrictions on the use of some of the preservatives (Rule 52 to 55B).

Part XI – deals with the crop contaminants and naturally occurring toxic substances such as Aflatoxin, Agaric Acid, Hydrocynic Acid, and Saffrole (Rule 57A and 57B).

Part XII – deals with the use of anti-oxidants, emulsifying/ stabilizing and anti-caking agents. Under this chapter, the definition of anti-oxidants, emulsifying and stabilizing agents along with maximum limit of these additives, which can be used in food products, have been prescribed. (Rule 58 to 62B).

Part XIII – deals with the flavouring agents and related substances.

Part XIV – deals with the use of insecticides and pesticides in food products. Direct use of pesticides on food is prohibited. This part also lays down the tolerance limit of various pesticides in different food products. (Rule 65).

Part XV – Solvent extracted oil and edible flour: deals with the products which are manufactured by Solvent Extraction Process. Only n-Hexane (food grade) shall be used as solvent. The limits of n-Hexane in food has been prescribed.

Part XVI – Sequestering and Buffering Agents: deals with sequestering and buffering agents and lays down the definitions of these agents. Under this part, a list of these agents has been given with the maximum level, which can be used in different food products (Rule 70 to 72).

Part XVII – deals with the Irradiation of food. Under this part, around 13 category of food products have been permitted for irradiation.

Appendix 'A': Different forms used by Food Inspectors, Public Analysts, Central Food Laboratories, have been informed. (Form I to VIII).

Appendix 'B': Under PFA Rules, specifications of various food products have been laid down which are in Appendix 'B' to the Rules. The food products which have been standardized are Carbonated Water (A.01), Baking Powder (A.02), Starchy foods (A.03), Spices & Condiments (A.05) Bean (A.06), Sweetening Agents (A.07), Coffee (A.08), Edible Fat (A.10), Milk & Milk products (A.11), Margarine (A.12), Tea (A.14), salt (A.15), Fruits and Vegetable products (A.16), Edible Oils (A.17), Cereals & Cereal products (A.18), Vanaspati (A.19), Vinegar (A.20), Catechu (A.21), Gelatin (A.22), Sweets and Confectionary (A.25), Food colours (A.26), Silver leaf (A.27), Groundnut Kernel (A.28), Alcoholic Beverages (A.29), Pan Masala (A.30), fat Spread (A.31), Mineral Water (A.32), Packaged Drinking Water (A.33) and Packed Meat and Meat products (A.34).

Under Appendix 'B' most of the foods, which are in market generally, are covered, but still there are large number of foods, which are not standardized. Such products are treated as proprietary food under Rule 37A.

From the foregoing, it is proved that the Food Laws in India are comprehensive covering all aspects.

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Why national food legislation is required?

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2. List the common food hazards.

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3. List the salient features of the PFA Act.

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4. What is an adulterated food under the PFA Act?

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2.3.2 Fruit Product Order, 1955

The Fruit Product Control Order was issued in 1946. Subsequently, this Order was brought under Essential Commodities Act, 1955 and thereafter this Order is known as Fruit Products Order, 1955.

The Ministry of Food Processing Industries, Govt. of India, administrates this Order. The Order provides for compulsory licensing for manufacturing fruit and vegetable products.

To ensure hygienic conditions of the manufacturing unit and its workers, the Order lays down the hygienic and sanitary requirements for setting up factories for the manufacture of fruit and vegetable products. It is also essential to have a laboratory in the manufacturing unit to test the quality and specification of the products.

Under this Order, specifications of various fruit and vegetable products have been laid down. These specifications are at least equal to the specifications laid down under PFA Act if not higher.

2.3.3 Vegetable Oil Products (Regulation) Order, 1998

The Vegetable Oil Products (Regulation) Order has been issued in 1998 in super session of Vegetable Oil Product Control Order, 1947 which was issued under Section 3 of Essential Supplies Act, 1946, in super session of Vegetable Oil Products Control Order, 1946 which was in existence at that time. Later on, after passing the Essential Commodities Act, 1955, the Vegetable Oil Product Order was adopted under Essential Commodities Act. This Order is implemented by Directorate of Vanaspati, Vegetable Oils & Fats, Ministry of Food & Civil Supplies. Govt. of India. Products covered under this Order are Vegetable Oils, Vanaspati, Margarine and Bakery & Shortening. The Order provides for compulsory licensing for manufacturing units. The specifications of the products, namely, Vanaspati, Margarine and Bakery & Shortening have been laid down under this Order.

2.3.4 Solvent Extracted Oil, De-Oiled Meal and Edible Flour (Control) Order, 1967

This Order issued under Section 3 of Essential Commodities Act, 1955, for controlling the production and distribution of Oils, De-oiled Meal and Edible Flour, which are obtained by the methods of solvent extraction. This Order provides for compulsory licensing of manufacturing units. The licence to such units under Rule 50 of the PFA Rules is exempted. The specifications of the edible oils produced by solvent extraction method have been laid down under the said Order. The packing & labelling conditions of such oils are also laid down but these oils shall conform to the specifications laid down under item A.17 of Appendix ‘A' of the PFA Rules.

2.3.5 Meat Product Control Order, 1973 (Ministry of Rural Areas and Employment)

This order has been issued under the essential commodity act, 1955. Licensing under PFA Rules, 1955 are exempted for those industries which manufacture meat and meat products. Quality and safety parameters have been prescribed for meat and meat products under this order. The quality and safety parameters have now been provided under PFA Rule, 1955 for these products also. However, under this order, requirement of Heavy metals and Pesticide Residues have been prescribed. Additionally, the list of preservatives, sequestering and buffering agents for use in these products have also been provided. This order emphasizes for the hygienic conditions of the plant machinery and personnel. Agricultural Marketing Advisor to Government of India is implementing this order.

2.3.6 Edible Oil Packaging (Development and Regulation) Order, 1998

Ministry of Consumer Affairs has issued this order in 1998 during the period of dropsy in India. In 1998, some mustard oils were found to be adulterated with argemone oil. To control the quality of edible vegetable oils the above order was issued. The main provision of this order are as follows:

- All edible vegetable oils shall be sold only in packed conditions.
- All the edible vegetable oils shall be packed only after testing by the manufacturers.

- All the edible oils shall meet the requirement prescribed under PFA Rules, 1955 for such oils.

- The methods of analysis to be used are the same, which have been prescribed in the manual prescribed by Directorate General of Health Services.

- All the manufacturers will have to register themselves with the local civil supply departments and shall file the returns as prescribed under that order.

2.3.7 Milk and Milk Product Order, 1992, Ministry of Agriculture

This order was issued under the essential commodities act, 1955. The Ministry of Agriculture, Department of Animal Husbandry and Dairying, Government of India are implementing this order. Under this order, conditions for registration and its renewal have been prescribed. The concerned inspector assesses the hygienic conditions of the premises and the quantity of such product being processed. Quality and safety parameters for milk and milk products have not been prescribed under this order meaning thereby that all the milk and milk products shall meet the Standards prescribed under PFA Rule, 1955. The main aim of this order is to control the collection of milk and production of milk products in addition to hygienic requirement of the plant, machinery and personnel.

2.3.8 Standard of Weights and Measures Act, 1976

The Standard of Weights and Measures Act, 1976 was enacted to establish standards of weights and measures, to regulate inter-state trade or commerce in weights, measures and other goods which are sold or distributed by weight, measure or number, and to provide for matters connected therewith or incidental thereto. The Act extends to the whole of India.

When commodities are sold or distributed in packaged form in the course of inter-State trade or commerce, it is essential that every package must have:

- Plain and conspicuous declaration thereon showing the identity of the commodity in the package,

- The net quantity in terms of the standard units of weights and measures and if in nos., the accurate number therein,

- The unit sale price of the commodity and the sale price of that particular package of that commodity.

- The names of the manufacturer, and also of the packer or distributor, should also be mentioned on the package.

In this regard the Packaged Commodities Rules were framed in 1977. These Rules extend to the whole of India and apply to commodities in the packaged form which are, or are intended or likely to be sold, distributed or delivered or offered or displayed for sale, distribution or delivery or which are stored for sale, or for distribution or delivery in the course of inter-state trade and commerce.

2.3.9 Export (Quality Control and Inspection) Act, 1963

The Export Inspection Council (EIC) was set up by the Government of India Export (Quality Control and Inspection) Act, 1963 (22 of 1963), in order to ensure sound development of export trade of India through Quality Control and Inspection and for matters connected thereof. The Council, constituted by the Central Government, is the apex body and has powers to constitute specialist committees to assist it in discharge of its functions. Accordingly, the Council has constituted Administrative Committee to advise it on administrative matters and a Technical Committee to advise it on technical matters.

Besides its advisory role, the Export Inspection Council, also exercises technical and administrative control over the five Export Inspection Agencies (EIAs) at Chennai, Delhi, Kochi, Kolkata and Mumbai. EIA's were established by the Ministry of Commerce, Government of India, under for the purpose of implementing the various measures and policies formulated by the Export Inspection Council of India.

Further, under the Export (Quality Control and Inspection) Act, 1963 following units have been set-up by the Ministry of Commerce for ensuring promotion and quality control of export of food item:

- Export inspection Council. (E.I.C.);
- Agricultural and Processed Food Export Development Authority (APEDA);
- Spices Board;
- Coffee Board;
- Tea Board;
- Marine Products Export Development Authority (MPEDA).

Pre-shipment inspection and analysis is carried out in order to ensure that exported items conform to the quality prescribed by the importing countries and do not pose any health hazard.

In case of some of the food article like spices and condiments, fruit products, and meat products, the system of compulsory certification has been introduced.

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Describe briefly the provisions of Fruit Products Order, 1955.

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2. Which are the important provisions of 'Solvent extracted oil, De-oiled Meal & Edible Flour (Control) Order, 1967 and 'Standard Weights & Measures Act, 1976?

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In addition to the above legislations, there are a few voluntary based product certification agencies in the country viz. BIS, and Agmark.

2.3.10 Bureau of Indian Standards Act, 1986

Bureau of Indian Standards has been constituted under an act of Parliament i.e. BIS Act, 1986, which deals with standardization of various consumer goods including food products.

The organization also runs a voluntary certification scheme known as "ISI Mark for certification of processed food items". The authorities after inspection and ensuring that the manufacturers have got the necessary technical know-how, hygienic conditions and other facilities available, grant them a certificate to use ISI mark on their products. Under the provisions of PFA Act, it has been made compulsory that commonly used food additives permitted for use in specified items of food, condensed milk, different categories of Milk Powder, Infant milk substitute, Infant food, packaged drinking water and mineral water and some food additives will be sold only under ISI Certification Mark.

2.3.11 Agmark Grading and Marking Act and Rules, 1937

Under the Grading and Marking Act the Directorate of Marketing and Inspection was constituted in the Ministry of Rural areas and Employment, which operates a voluntary scheme of certification of agricultural products (raw and processed) for safeguarding the health of consumers under 'Agmark'. An approved chemist tests each batch of consignment before certification is granted. The PFA Rules, 1955 provide compulsory Agmark certification of Blended Oils, *Carbia callosa* and Honey dew; Kangra tea; Ghee moving from one state to another; Til Oil produced in Tripura, Assam and West Bengal. Further, certain food items meant for export have been brought under compulsory Agmark certification viz. walnuts, black pepper, cardamom, chillies, ginger and turmeric etc.

The Directorate of Marketing and Inspection has 21 laboratories and 50 sub offices spread all over the country. The Central Agmark Laboratory at Nagpur carries out research and development work in this field.

Check Your Progress Exercise 3

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Name two voluntary certification agencies.

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2. Explain 'ISI mark and 'Agmark'.

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2.4 FOOD REGULATIONS FOR INTERNATIONAL ORGANIZATIONS

Since ancient times authorities all over the world made attempts to codify the tools to protect the consumers from dishonest practices in sale of foods. With development in food science, food chemistry and consumer awareness, national governments and international organizations stepped into food and health area, to protect consumers from fraud and to provide clean, wholesome, nutritious and safe food to all.

2.4.1 Codex Alimentarius Commission (CAC)

An UN resolution passed in 1963 to establish Joint FAO / WHO programme paved way for CAC's Food Standard Programme.

During the past 4 decades, all aspects of foods – processing, labelling, packaging, nutrition, consumer health and fair practices in trade have come under the commission's scrutiny.

So far Codex has:

- Formulated standards for 237 food commodities.
- Formulated 41 codes of hygienic practices.

- Evaluated 185 pesticides.
- Prescribed limits for contaminants.
- Developed guidelines for 25 contaminants.
- Evaluated 1005 food additives.
- Evaluated 54 veterinary drugs.

Codex India is the National Codex Contact Point (NCCP) for India, it is located at the Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi. It coordinates and promotes Codex activities in India.

2.4.2 International Organization for Standardization (ISO)

The objective of ISO is to promote the development of standards in the world with a view to facilitate international exchange of goods and services, and to develop mutual co-operation in the sphere of intellectual, scientific, technological and economic activity. Other functions of ISO are:

- Helps in Harmonization of food standards throughout the world.
- Facilitates exchange of scientific knowledge between countries.
- Promotes economic development.
- Promotes exchange of goods.
- Promotes free and fair global trading with strategic partnership with WTO.
- Standards help to revise levels of quality, safety, reliability, efficient, compatibility and inter-exchangeability.
- Safeguards consumers and users.
- Global exchange of goods and services incorporating rationality, practical applicability, environmental protection, safeguards safety and health, and provides equal opportunities in world trade.

Check Your Progress Exercise 4

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Explain the role of Codex Alimentarius Commission.

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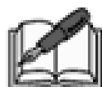

2.5 LET US SUM UP

Food quality and safety are of paramount importance. Each country has evolved its own food legislations to safeguard the health and well being of its citizens and also promotes its international trade in raw and processed foods. India has enacted the food legislation called the Prevention of Food Adulteration Act (PFA). Besides PFA, few other Acts and Orders of the Government of India deal with specific food commodities. Certification of quality by BIS and Agmark also ensures food product and fresh produce quality.

International food standards like CODEX play very important role in international trade of fresh and processed foods. National food standards are being harmonized with the international standards to further strengthen world trade in food and food products.

2.6 KEY WORDS

PFA : Prevention of Food Adulteration Act, 1954.

FPO : Fruit Product Order, 1955.

ISI : Indian Standards Institution.

BIS : Bureau of Indian Standards.

ISO : International Organization for Standardization.

2.7 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:
   - Food borne illnesses
   - Adulterated food
   - Domestic and international trade
2. Your answer should include the following points:
   - Microbial
   - Pesticide residues
   - Misuse of food additives
   - Metals, toxins
3. Your answer should include the following points:
   - Definition of adulteration
   - Public analysts and food inspectors
   - Consumer organizations, legal proceedings
   - Imported foodstuffs
   - Warranty
   - Trial and punishment

4. Your answer should include the following points:

   - Not meeting specifications
   - Containing injurious substances
   - Addition of cheaper, inferior substances
   - Food prepared under insanitary conditions
   - Unfit for human consumption

Check Your Progress Exercise 2

1. Your answer should include the following points:

   - Ministry of Food Processing Industries
   - Hygienic and sanitary conditions in factories
   - Specifications of fruits and vegetable products
   - Compulsory licensing

2. Your answer should include the following points:

   - Compulsory licensing solvent extraction units
   - Specifications of oils
   - Packaging and labelling
   - Quality and safety of meat products
   - Heavy metal and pesticide residues
   - Preservatives, sequestering and buffering agents

Check Your Progress Exercise 3

1. Your answer should include the following points:

   - FAO/ WHO
   - International food standards
   - Standards for food commodities
   - Codes of hygienic practices
   - Limits for food contaminants
   - Food additives

2. Your answer should include the following points:

   - ISI mark is covered under Bureau of Indian Standards Act, 1986
   - Certification Mark of quality
   - Standardization of goods including food products
   - To ensure production of food products under hygienic conditions
   - Under PFA Act food additives, specific food and drinking water and mineral water will be sold under ISI certification Mark
   - Agmark a voluntary scheme of certification of agricultural produce
   - It is operated under Agmark Grading and Marketing Act and Rules, 1937
   - Agmark ensure for safeguarding the health of consumers
   - Blended oils, Ghee, Til oil, etc. are sold under Agmark certification

Check Your Progress Exercise 4

1. Your answer should include the following points:

   - Codex Alimentarius Commission (CAC) operates under Joint FAO/WHO Programme
   - It scrutinized Food Standard Programme
   - Formulate standards for food commodities, hygienic practices, pesticides residues, food additives etc.

2.8 SOME USEFUL BOOKS

1. Prevention of Food Adulteration Act (1954) 24th Edition, 2003, Easterb Book Company, Lucknow (www.nohfw.nic.in/pfa).

2. Fruit Products Order (1955) All India Food Preserver's Association, New Delhi. (www.mofpi.nic.in).

UNIT 3 FOOD SAFETY MANAGEMENT

Structure

3.0 Objectives
3.1 Introduction
3.2 Food Safety
    Food Hazards
    Importance of Safe Foods
3.3 Food Safety Programmes
    Good Manufacturing Practices (GMP)
    Hazard Analysis Critical Control Points (HACCP) System
    International Organization for Standardization (ISO)
    Total Quality Management (TQM)
3.4 Let Us Sum Up
3.5 Key Words
3.6 Answers to Check Your Progress Exercises
3.7 Some Useful Books

3.0 OBJECTIVES

After reading this unit, you should be able to:

- explain the importance of food safety and understand different food safety programmes like:
    - good manufacturing practices;
    - hazards analysis and critical control points;
    - international organization for standardisation; and
    - total quality management.

3.1 INTRODUCTION

The demand for fresh and processed foods throughout the world is increasing steadily along with the increasing population. This has necessitated bulk handling, processing, storage and distribution of foods. There is every possibility that some times these operations are carried out defectively introducing food hazards. The developments in food science have opened up the possibility of using numerous food additives capable of preserving and modifying foods to the requirements of the consumer. However, inadvertent use of these additives can also result in food hazards.

The conventional quality control methods in which the quality of the fresh or processed foods is tested just before distribution, though has been very useful in ensuring food safety, is a post-mortem exercise. This means that if the food at the distribution stage is found to be defective, there is no way to salvage it. In order to overcome this drawback, new Food Safety Management Systems have evolved. These systems, besides ensuring food safety, also enable production of food products with no or minimum defects. You will be learning the basic aspects of some of these systems in this unit.

3.2 FOOD SAFETY

Food is comprised of an array of chemicals, namely; proteins, fat, carbohydrates, vitamins, minerals and fibre which are required to sustain life. These constituents of food have nutritional value. We all expect food to be nutritious, wholesome, and safe. **Absolute safe food** is the one, which will not cause any damage or harm. However, our food is subject to contamination and therefore, **relative food-safety** can be defined as the practical certainty that injury or damage will not result from a food used in a **reasonable** and customary manner and **quantity**.

Food safety can be understood in a better way if we use two basic concepts – Toxicity and Hazard. Toxicity is the capacity of a substance to produce harm or injury. Hazard is the relative probability that harm or injury will result when the substance is used in a proposed manner and quantity.

3.2.1 Food Hazards

You have already learned some aspects of food hazards in an earlier unit. A hazard is a biological, chemical or physical agent in a food, which has the potential to cause harm or injury to the health.

Biological hazards include pathogenic bacteria, fungus, virus and parasites and toxins elaborated by these organisms. They may cause infections and produce toxins.

Chemical hazards include naturally occurring toxicants such as trypsin inhibitor, solanins, haemagglutinins, phytates, cyanogenic glycosides and alkaloids; heavy metals such as lead, cadmium, arsenic and mercury; pesticide residues like DDT, malathion, parathion, endosulfan, etc. Chemical hazards can also be mycotoxins like aflatoxins developed on nuts and corns, veterinary drug residues and also unapproved food additives or additives added in excess.

Physical hazards include extraneous matter such as stones, glass fragments, dirt, metal bits, etc.

3.2.2 Importance of Safe Foods

A safe food ensures prevention of food borne diseases, and provides nutrition and good quality to the consumer. It also promotes international trade and stimulates economic development.

Maintaining food safety and quality is essential in the entire chain of food production ranging from raw agricultural commodity at farm level; primary food processing at the farm, dairy, abattoir (slaughter house) and grain mills; secondary food processing level such as canning, freezing, drying and brewing and packing; food distribution both at national and international level; food retailing and food catering and domestic food preparation.

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Differentiate between absolute and relative food safety.

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2. Explain different types of food hazards.

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3.3 FOOD SAFETY PROGRAMMES

Consumer confidence in the safety and quality of the food supply is an important requirement. A successful safety programme involves a shared responsibility among Food Industries, Government and Consumer. Food Safety Management has progressed rapidly in recent years. The international agencies like Food and Agriculture Organization (FAO) and World Health Organization (WHO), Codex Alimentarius Commission (CAC) – a joint FAO / WHO programme, and International Organization for Standardization (ISO) are playing vital role in the safety management of foods.

3.3.1 Good Manufacturing Practices (GMP)

Good Manufacturing Practices can achieve food safety. Good Manufacturing Practices in manufacturing and packing are pre-requisites for acceptable food safety. GMPs are essential for the manufacture and distribution of foods that are safe from microbiological, chemical, and physical hazards. It is essential that the food industry manage a comprehensive programme that evaluates, identifies, and controls potential hazards at every step in the production, development and manufacturing environment.

Requirements for GMP for Food Industry

Good Manufacturing Practices are prescribed to ensure that:

- Factory is at proper location.
- Factory has right layout and building design.
- Raw materials used in the products are of right specifications.
- Manufacturing processes are properly prescribed and implemented to ensure right quality finished products.
- Adequate quality standards are in place.
- All critical control points are specified by hazard analysis.
- Finished products are released for market only after prescribed quality analysis.
- These are stored and transported in hygienic manner.
- All market returns are properly stored, analyzed, reworked or disposed off with proper procedure.
- Traceability procedure is in place.

To achieve the above objectives each manufacturer ensures that each step is properly followed with detail procedures in place.

3.3.2 Hazard Analysis and Critical Control Point (HACCP) System

Hazard Analysis Critical Control Point (HACCP) system is a prevention system. Here the focus for control is on the manufacturing process. Various monitoring and control methods are applied to reduce or eliminate the possibility of contamination. HACCP is a worldwide – recognized systematic and preventive approach that addresses biological, chemical and physical hazards through anticipation and prevention during manufacturing process.

Important Definitions

Hazard: the potential to cause permanent or temporary injury to a consumer

Severity: The magnitude of consequences resulting from a hazard.

Risk: An estimate of the probability of a hazard occurring.

Control: Actions taken or conditions applied either to reduce to acceptable levels or to eliminate a hazard.

Critical Control Point: A point in the food manufacturing, distribution and use chain where control is exercised.

Benefits of HACCP Certification

- It will reduce the risk of customers being food poisoned.
- It will increase quality of the product.
- It will ensure compliance with the law.
- It will reduce reliance on end-product inspection and testing.

Principles of HACCP

Food Safety Management System by HACCP has seven principles:

1. Identify the hazards:

   Look at each step (e.g., purchasing, delivery, storage, preparation, cooking, serving and display, etc.) in your operation and identify what can go wrong.

2. Determine the Critical Control Points (CCPs):

   Identify the points in your operation that ensures control of hazards, e.g., adequate cooking will kill E.coli and other pathogens.

3. Establish Critical Limits:

   Set limits to enable you to identify when a CCP is out of control, e.g., the critical limit for hot holding of cooked foods is +63°C.

4. Establish a system to monitor control of CCP you should decide

   - Who should check that the critical limit has not been exceeded,
   - How often the check should be done, and
   - What exactly the check involves.

5. Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.

   When monitoring indicates that a CCP is not under control, corrective action must be taken, e.g.

   - Discard the food when it is past the use before a particular date

6. Establish procedures for verification to confirm the HACCP system is working effectively. Review and correct the system periodically and whenever you make changes to your operations, e.g.,

   - Change of recipe, installation of new equipment, etc.

7. Establish documentation concerning all procedures and records appropriate to these principles and their application.

   For the successful implementation of the system, appropriate documentations and records must be kept and be readily available, e.g., - Temperature record sheets.

A safety food management system based on these principles will enable hazards to be identified and controlled before they threaten the safety of the food served to customers and damage the reputation.

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. List the requirements of GMP for food industry.

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2. Define 'Hazard' and 'Critical Control Point'.

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3. List the benefits of HACCP certification.

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4. List the principles of HACCP.

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3.3.3 International Organization for Standardization (ISO)

The global market place provides opportunities for food processors and also creates concerns for consumers. International standards provide tools to reduce consumer concerns and provide tools for the promotion of food trade.

The International Organization for Standardization (ISO) is a non-governmental organization located in Geneva, Switzerland. It was formed in 1947 to develop a common set of manufacturing and trade and standards to facilitate international trade. ISO is made up of 138 nations.

ISO: 9000 – 1994 is an international standard directed at the quality management process of an organization; it included the group of standards ISO-9001, ISO-9003 and ISO-9004.

ISO-9001 standard is a management tool that focuses on meeting the customer's needs and expectations; every step in achieving the quality is documented. The documented system defines policies, objectives, and expected performance.

"Quality Management" refers to "all activities of the overall management function that determines the quality policy, objectives and responsibilities of the quality system".

Quality System is "organizational structure, procedures, processes and resources needed to implement the quality management. The ISO-9001: 1994 standard focuses on the existence, implementation, and effectiveness of the quality system as a whole. ISO-9001: 2000 is the latest Quality Management system.

ISO Certification is provided to the organization that has a quality management system that meets the scope of the stated standard.

Certification is the procedure by which third party gives assurance that a product, process, or service conform to specific requirements. [First party is the manufacturer and second party is the consumer. Here third party is ISO certifying agency.

Benefits of ISO Certification

Improved efficiency through both documentation and communication

- Improved consistency of manufactured items.
- Reduction in amount of re-work and non-conforming product.
- Improved customer satisfaction.
- Improved motivation and employee involvement through all levels of process.
- Reduced customer complaints.

3.3.4 Total Quality Management (TQM)

Japanese designed and built goods such as motorcars; cameras, radio and TV sets have quality and reliability. It is because Japanese industry has the ability to cope up with change and accordingly improved their management skill by adopting Total Quality Management System (TQM).

Definition: TQM is the application of quantitative methods and human resources to improve:

i) The material and services supplied,
ii) All the processes within the organization, and
iii) Degree to which the needs of the customers are met.

The TQM is a process and a journey and continuous; it is not a destination. It is a philosophy, culture and a way of doing business.

Basic Tenets of TQM

- Focus on customer satisfaction,
  - Internal customers
  - External customers
- Continuous improvements,
- Employee investment and empowerment,
- Measurement and documenting the work,
- Doing it right the first time,
- Effective communication, education and training,
- Leadership from top,
- Providing everyone with the opportunity to do their job properly.

Benefits of TQM

- Improvements in leadership qualities and more visible leadership from executives and senior managers.
- Involving personnel in decision making process.
- Increased confidence of personnel in their ability to carry out their work and to achieve targets.
- Reduction of mistakes, increased pride in work, sense of achievement for workers.
- Opportunity for self-development and self-improvement of personnel through a pro-active involvement in work.
- Opportunity to engage in creative thinking to improve product quality and work environment.
- Increased co-operation quality and work environment.
- Increased co-operation, improved teamwork and reduced conflict.

Check Your Progress Exercise 3

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. List the benefits of ISO certification.

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2. Define TQM.

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3. List the benefits of TQM.

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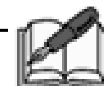
3.4 LET US SUM UP



Ensuring the safety of fresh and processed foods is the primary responsibility of all those involved in food handling and food processors. In order to achieve that the industry has been relying on quality control methods since long time. Although quality control has been successful in ensuring food safety and quality, it has certain drawbacks. Since it is carried out mostly at the end of the manufacturing process, retrieval of defective products is rather impossible. Rejection of defective products above a certain level adversely affects the economics of the industry. Besides, certain hazards entering the food processing operations are difficult to detect by routine analysis. Therefore, certain food safety programmes have evolved, which are capable of ensuring

virtually zero levels of defects and hazards. The maim advantage of such programmes is the possibility of tracing the cause of defects easily based on the documentation, so that such recurrences can be averted.

3.5 KEY WORDS

Biological hazards : Pathogenic bacteria, fungus, virus and parasites and toxins elaborated by these organisms.

Chemical hazards : Naturally occurring toxins in foods, pesticide residues etc.

Physical hazards : Extraneous matter.

GMP : Good Manufacturing Practices.

HACCP : Hazard Analysis and Critical Control Points.

ISO : International Organization for Standardization.

TQM : Total Quality Management.

3.6 ANSWERS TO CHEECK YOUR PROGRAESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:
   - Will not cause any harm
   - Practical certainty of no harm
2. Your answer should include the following points:
   - Biological
   - Chemical
   - Physical

Check Your Progress Exercise 2

1. Your answer should include the following points:
   - Proper factory location
   - Proper layout
   - Good raw materials
   - Quality standards
   - Quality analysis
   - Traceability
2. Your answer should include the following points:
   - Permanent or temporary injury
   - Point where control is exercised
3. Your answer should include the following points:
   - Reduce risk of food poisoning
   - Increase quality

- Compliance with law
- Reduce reliance of end product analysis

4. Your answer should include the following points:

- Identify hazards
- Determine CCPs
- Critical limits
- Corrective action
- Verification
- documentation

Check Your Progress 3

1. Your answer should include the following points:

- Improved efficiency
- Consistency
- Deduced non conformity
- Customer satisfaction
- Less customer complaints

2. Your answer should include the following points:

- Application of quantitative methods and human resources to improve
- Materials and services supplied
- Processes within the organization
- Needs of customers

3. Your answer should include the following points:

- Leadership
- Decision making
- Confidence
- Sense of achievement
- Self- development
- Product quality
- Team work

3.7 SOME USEFUL BOOKS

1. Gould and Gould, Total Quality Assurance for Food Industries, CTI Public. Inc., Baltimore, USA

2. Ralph, Early (1955) Guide to Quality Management Systems for Food Industry, 1st Edition, Blackie Academic Professional, London

UNIT 4 TESTING AND EVALUATION: PHYSICAL METHODS

Structure

4.0 Objectives
4.1 Introduction
4.2 Colour
Factors Affecting the Colour of Objects
Approaches to Colour Measurement
Colour Matching
Quantitative Measurement of Colour
The CIE system
Hunter Colour System and Colour Difference Meter
4.3 Viscosity and Consistency
Flow Behaviour of Fluids
Bostwick Consistometer
Brookfield Synchrolectric Viscometer
4.4 Texture
Magness-Taylor Pressure Tester
Instron Testing Machine
Measurement of Jellying Property of Pectins
4.5 Let Us Sum Up
4.6 Key Words
4.7 Answers to Check Your Progress Exercises
4.8 Some Useful Books

4.0 OBJECTIVES

After reading this unit, you should be able to:

- understand colour in quantitative basis;
- describe methods for measurement of colour;
- understand viscosity, consistency and texture of foods; and
- describe methods for their measurement.

4.1 INTRODUCTION

Physical methods are used for both analysis and quality control. Analytical methods based on physical properties of food constituents are covered in detail under "Instrumental methods of analysis" in Block 7.3. In this unit, some of the important quality control parameters such as colour and Texture are described.

4.2 COLOUR

We associate colour and appearance of food with its quality. Quite often, colour plays the dominant role in assessing the overall quality of a food material. Examples are colour of fruits, vegetables, sweet meats, bakery products, ice creams and so on. Colour changes in foods during processing and storage are common. Change of the green chlorophyll colour of vegetables,

browning reactions etc. is examples. In order to make foods more attractive to the consumer, natural and artificial colorants are some times added.

What is colour? Why an object is red, yellow or green? Colour is an appearance property attributable to the spectral distribution of light. Light as we commonly refer to is that part of the electromagnetic radiation to which the human eye is sensitive. The radiations of different wavelengths are called the spectrum of the radiation. The visible region of the spectrum is only a very minute part of the electromagnetic spectrum in the range of 380 to 780 nm. You will be learning more about the properties of electromagnetic radiation in the unit on 'Instrumental methods of analysis'.

As you may know, visible light can be split into its spectrum by passing it through a prism. The spectrum consists of the colours red, orange, yellow, green, blue, indigo and violet. If the light radiation striking the retina of the eye does not contain all the wavelengths of the visible spectrum, or if their intensities differ considerably, the sensation of colour results. This happens because when light radiation strikes an object, it may interact with the object in different ways depending on the nature of the object, one being absorption of the radiation. The absorption of all the wavelengths of the radiation may not take place uniformly. As a result, some wavelengths are reduced in intensity and the resultant radiation having certain dominant wavelength of the radiation is either reflected (opaque medium) or transmitted (transparent medium). The reflected or transmitted light is perceived as the colour of an object by the eye. Measurement of the transmitted radiation is the basis of spectrophotometry, which you will be learning in the next block. The measurement of the reflected light radiation is the basis of objective measurement of colour of objects like food products.

4.2.1 Factors Affecting the Colour of Objects

The perceived colour of an object in terms of its shade, brightness etc. is dependent on three major factors:

i) the chemical and physical nature of the object;
ii) the spectral power distribution in the light from the light source;
iii) the sensitivity perception system.

The colour of an object is primarily dependent on the colour producing chemical substances present in it. However, the shade, brightness and appearance of the colour can be greatly affected by its physical form. For example the colour of a whole apple is different from the crushed apple, or the colour of roasted coffee beans is different from ground coffee. In case of liquid foods, the colour varies with the depth of the solution (light path).

The perceived colour of an object is also dependent on the light illuminating it. A colour may appear dull in dim light but bright in bright light. The colour also depends on the spectral distribution of the light. The colour of an object viewed under a coloured light is different from the colour under white light.

The object properties and illumination interact to provide the stimulus for the receptor mechanism i.e. the eye and the brain system to perceive the colour. Perception of colour is unique to the individual. They change with colour vision abilities. Approximately 8% of the population perceive colour in a different way from the remainder. The other 92% of the population do not perceive colour in exactly the same way.

The above factors make it necessary to evolve an agreed terminology for colour description and measurement. Colour measurement implies expressing the above concepts in numerical dimensions.

4.2.2 Approaches to Colour Measurement

Two approaches are possible for colour measurement. The simplest method is to use a numerical index, which defines a colour adequately for specific purposes and enables comparison. This could be termed '**colour comparison'.** Obviously, this method does not specify all the attributes of a colour, which is required for several purposes. The second approach is to quantitatively specify the colour by determining the recognizable attributes of the colour.

4.2.3 Colour Matching

The eye has tremendous capacity to discriminate colours. However the capacity of the brain for remembering them is poor. For example, the brain cannot exactly remember the colour of a fruit of a previous year. During storage studies of food products, its colour changes and the original sample with out the colour change may not be available for comparison. Therefore, matching the colour with a colour order system is followed. Colour dictionaries (atlases), disc colorimeters and tintometers are usually used for the purpose.

Colour dictionaries: Colour dictionaries usually consist of sets of colour charts grouped into different hues. In order to get reliable data, matching of colours should be done by individuals with normal colour vision using standard viewing and lighting conditions. One of the most popular colour atlases used for horticultural crops is the **Royal Horticultural Society (RHS) Charts.**

Another colour atlas, which used to be widely quoted in food industry, is the **Munsell System.** In the Munsell System, colour dimensions are Hue (H), Value (V) and Chroma (C). The Figure 4.1 below is a sketch of the Munsell colour solid.

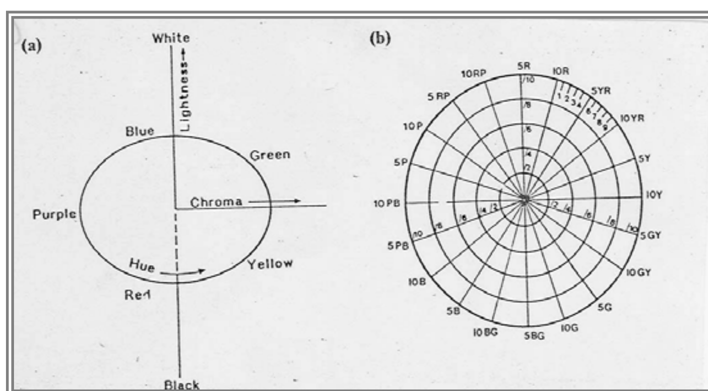


Figure 4.1: The Munsell colour system: a) dimensions of the Munsell colour system solid; b) organization of Hue and Chroma in the Munsell System solid.

The hue circle consists of ten major hues, each divided into ten equally spaced steps. The central achromatic Value (lightness) axis consists of ten equal steps, extending from ideal black = 0 to ideal white=10. The distance from this axis indicates an increase in Chroma that is an increase in hue content, and departure from grey. The Chroma is zero at the achromatic axis, and increases in visually equal steps to /10, /12, /14 or greater for particularly saturated

colours. The Munsell atlas consists of pages of coloured chips. The chips are arranged so that the vertical axis of the pages represents an increase in V, the horizontal axis an increase in C. The Munsell description of a yellow-red colour of hue 3YR, Value 5/, and Chroma 6/ is denoted as 3YR 5/6.

Munsell system is some times used in conjunction with Disc colorimeters like the Macbeth- Munsell Disc colorimeter. In the Disc colorimeter, rapid spinning mixes two or more colours in the form of interleaved discs. The resultant hue is the average hue of the sample, which is useful in certain situations like colour of vegetables or homogenised samples, but not for comparing non-uniform colour surfaces like the colour of fruits like apple.

Tintometers: The Lovibond Tintometer used to be widely used in the food industry for a long time before more refined instruments became available. It is still in use for specific applications. The instrument is provided with sets of red, yellow and blue glass slides as permanent standards. The slides form an evenly graded series from very light tints (0) to deep colours (20), numbered according to their depth of colour. The three series are so related that when three slides of equal value are combined and viewed against white, they appear grey or neutral in colour. With the help if an optical system in the instrument, the illuminated sample is made to occupy half the field of view while the other half receives reflected light from a standard white surface, which passes through the selected coloured glass. When the colour is matched, it is specified by the values of red, yellow and blue slides required as for example 14.0 R + 6.0 Y + 1.0 B.

4.2.4 Quantitative Measurement of Colour

A complete specification of colour requires measurement of three attributes of colour.

i) **Hue**: the kind of colour, red, blue or green.

ii) **Saturation**: the depth or strength of the hue or the extent to which the pure hue is mixed with white.

iii) **Lightness**: the extent to which the hue is diluted with black. It is associated with brightness of the hue.

The International Commission on Illumination (CIE: *Commission Internationale de l'Eclairage*, 1931) adopted a set of standards, which has made it possible to define the colour in absolute terms. The system is rather elborate and hence only some salient points are explained here.

4.2.5 The CIE System

The CIE system is based on the principle that any colour can be matched exactly by a suitable mixture of only three colours selected from the red or amber (R), green (G) and blue (B) parts of the light spectrum. The three colours are called "**primaries**" and their relative amounts required to match a colour are called "**tristimulus**" values of the colour. This postulate has been confirmed by colour matching using additive mixing of monochromatic lights of wavelength 650 nm (R), 530 nm (G), and 460 nm (B) to obtain colour matches by observers with normal colour vision called the standard observer. The amount C of each colour (C) is matched using amounts of R, G, and B of each particular stimulus (R), (G), and (B). i.e.

$$C\,(C) = R\,(R) + G\,(G) + B\,(B)$$

When C is unity:

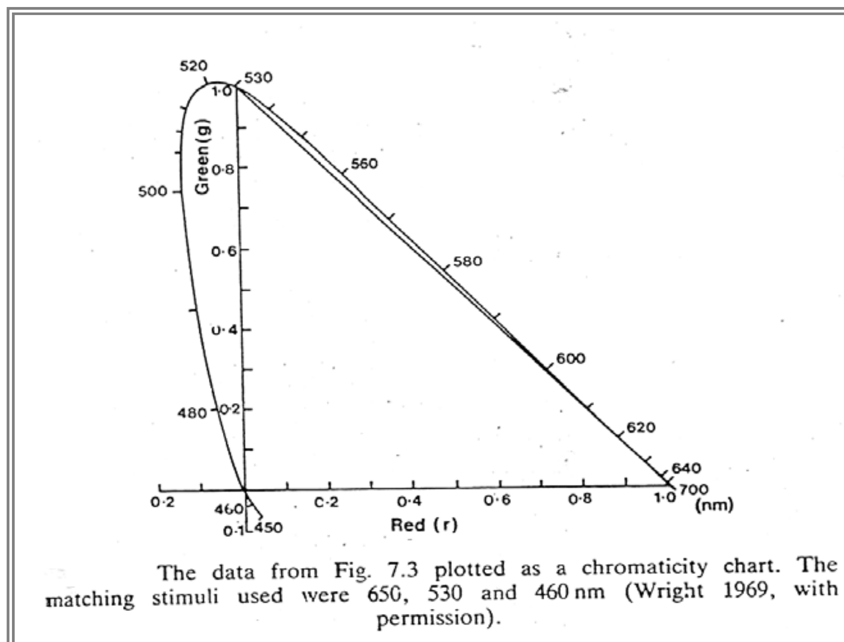
$$1.0\ (C) = r\,(R) + g\,(G) + b\,(B)$$

where:

$$r = \frac{R}{R+G+B}, \quad g = \frac{G}{R+G+B}, \quad b = \frac{B}{R+G+B}$$

Where: r, g and b are chromaticity coordinates of the colour i.e., r+g+b = 1

Therefore, if any two of r, g and b are specified, the third can be calculated. Hence, the results can be shown in the form of the two-dimensional **chromaticity diagram** in which r and g are usually plotted as x and y axis (Figure below).



The data from Fig. 7.3 plotted as a chromaticity chart. The matching stimuli used were 650, 530 and 460 nm (Wright 1969, with permission).

Figure 4.2

The spectral colours, for which the wavelengths are noted on the diagram, are shown as spectrum locus. The blue of wavelength approximately 460 nm, near one end of the locus is at the origin where r and g are zero and b = 1. The locus progresses to a wavelength of 530nm, where r is zero and g = 1 (and b is therefore zero).

It can be seen from the above figure that r, g and b have negative values. This is because the spectrum locus is convex and hence no real primaries exist which will always yield positive values. Therefore, CIE decided to use three unreal primaries (X), (Y) and (Z) so that the chromaticity coordinates x, y and z will always be positive. The modified chromaticity diagram is called the **CIE Chromaticity Chart,** which is shown below. Any given colour to be described in CIE terms can now be located in the spectrum locus by the relative distances along the x and y coordinates, representing respectively the values of X and Y.

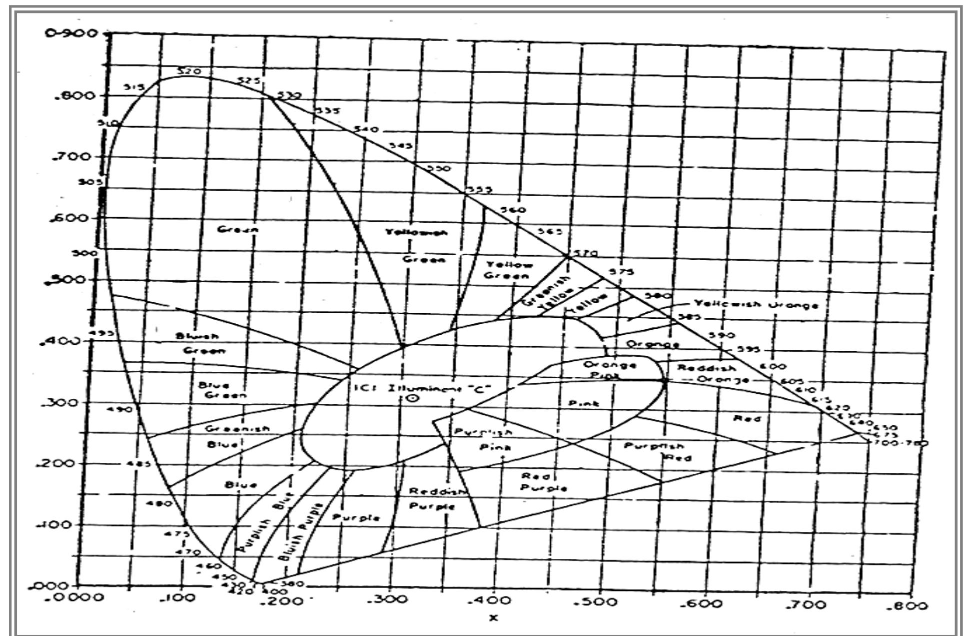


Figure 4.3: Chromaticity diagram

Having located a given colour in the Chromaticity chart from its chromaticity coordinates, it is necessary to find out its light intensity or brightness factor. This is done by assuming that all of the light energy represented by a colour is regarded as coming from Y stimulus. Therefore, the amount of Y is a direct measure of the brightness or lightness of the colour. If Y values are plotted perpendicularly to the chromaticity plane, the irregular colour solid is created within which any colour can be defined as a unique point with the CIE coordinates x, y and Y.

To determine the other visual dimensions of a colour, the colour (C) and the illuminant (S) used are marked on the chromaticity diagram as shown below.

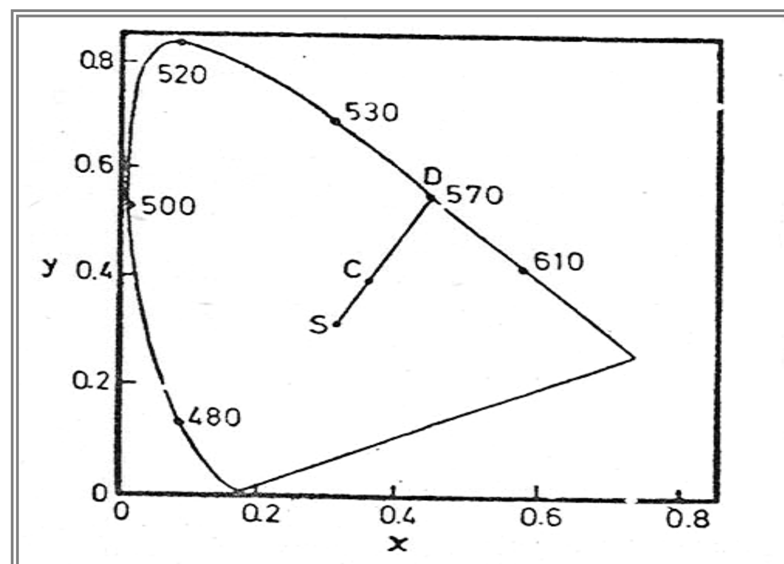


Figure 4.4: Determination of dominant wavelength and purity of colour

A line is drawn joining the two points and extrapolated to the spectrum locus (D), which is the **dominant wavelength** of the colour. Now the specification of the colour can be represented with reference of the above figure as:

Hue of the colour C is given in terms of the **dominant wavelength**

Saturation or chroma of the colour C is measured in terms of purity, which is the ratio of the distance SC to the distance SD. The ratio is usually expressed as percent.

Lightness of the colour C is given by its Y coordinate perpendicular to the chromaticity plane and is represented as Y%.

As mentioned earlier, the colour of an object is also dependent on the illuminant. Therefore, CIE system has defined three standard illuminants and their trichromatic coefficients. The illuminants are:

Illuminant A: Corresponds to the light from a gas filled tungsten lamp operated at a colour temperature of 2,856^{o}K.

Illuminant B: Corresponds to the more yellow type of average daylight, and consists of the standard illuminant in conjunction with a colour filter.

Illuminant C: Corresponds to light from the sky rather than sunlight. It consists of the illuminant A in conjunction with a different filter.

A spectrophotometric curve giving the intensities of light at different wavelengths of the visible region gives a complete specification of the colour. This can be calculated from the spectral data, which is quite tedious and hence not given here. This is made easy in the present day spectrophotometers, which automatically records the spectral curve and compute the CIE values Hue, Chroma and Lightness values.

4.2.6 Hunter Colour System and Colour Difference Meter

In the Hunter colour meter, tristimulus amber, green and blue filters together with suitable detection and measuring devices provide close approximation of the X, Y and Z functions of the CIE System. The Hunter colour space is slightly different to the CIE colour space (Figure below).

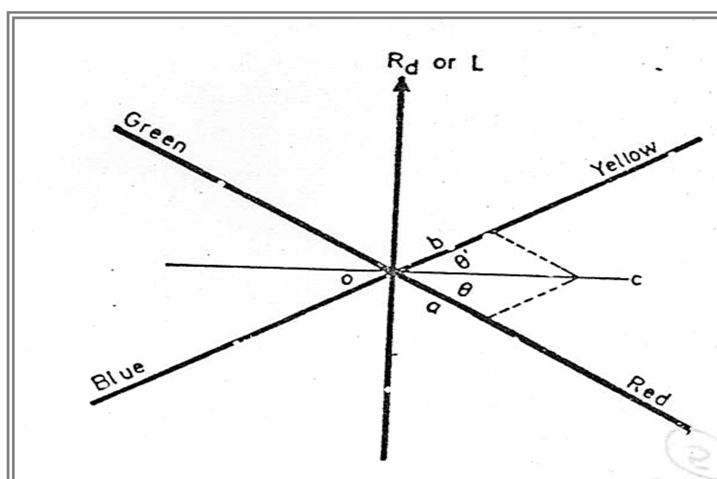


Figure 4.5: Hunter colour dimensions

The chromaticity plane is defined by dimensions *a* and *b*. The white point is at the origin. The Hunter positive *a* values indicate redness and negative *a* values greenness. The Hunter positive *b* values indicate yellowness and negative *b* values blueness. The *a* values are functions of x and y and *b* values those of Z and Y. For a particular colour C, hue or dominant wavelength is given by the ratio *a/b.* The saturation is given by the distance from the colour point C to the white point, which is $(a^2 + b^2)^{1/2}$. The Hunter R_d (diffuse reflectance) or visual lightness (L) is directly comparable to the Y of the CIE system.

Hunter values permit calculation of the colour difference (ΔE) between two colours like sample and standard colours. ΔE is given by:

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{½}$$

The modern Hunter colour difference meters are capable of providing outputs of the various colour parameters.

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. What is colour of an object?

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2. Which are the components of the complete specification of colour.

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3. Describe the CIE system of colour measurement.

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4. Describe the Hunter system of colour measurement.

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4.3 VICOSITY AND CONSISTANCY

The kinaesthetic attributes of food products, viz. viscosity, consistency and texture are perceived by human senses of sight, touch and mouth feel. We are able to judge whether a fluid food is thin or thick by watching how it flows, feeling it with fingers or by mouth feel. We decide the texture of fruits, vegetables etc. by finger feel. We also assess texture by biting or chewing. Obviously these assessments are subjective and tend to differ from individual to individual. Therefore, objective measurement of these quality attributes is important in quality control and product development.

Food products exist in various physical forms like thin liquids, viscous liquids, semi-solids and solids. You have observed that most of the thin liquids like beverages, milk etc. just flow out from containers. Products like tomato ketchup or sauces require some initial push (like hitting the bottom of the bottle) before they start to flow. Solids like fruits require much more pressure to compress and finally break (deformation). The study of flow and deformation of materials is called **rheology**.

Viscosity and consistency are flow properties of fluids while texture is the deformation property of solids. Obviously, the force required to initiate solid deformation is much higher than that required to initiate liquid flow. If the force required is 1.0 gravity or less, the term used is viscosity or consistency and when the force required is more than 1.0 gravity, the term used is texture.

4.3.1 Flow Behaviour of Fluids

Fluids (liquids) are classified as Newtonian or non-Newtonian depending on their flow behaviour. This can be explained easily with the help of a simple diagram.

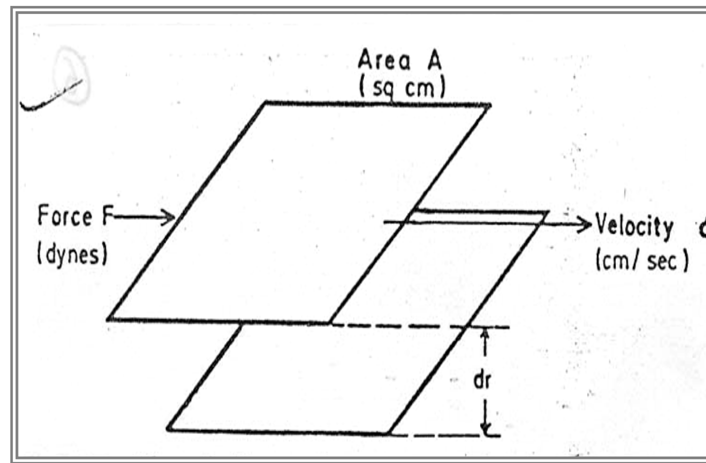

Figure 4.6: Schematic diagram illustrating the response of fluids to an imposed shearing force

Let us assume two layers of a fluid; each having an area of A cm^2 are separated by a distance dr cm. If the top layer moves parallel to the bottom layer at a velocity of du cm/sec relative to the bottom layer, a force of F dynes is required for maintaining the velocity. The velocity gradient represents the deformation, and is commonly referred to as the **shear rate**, which has the unit of per second. The shearing force per unit area (F/A) is the **shear stress** and is denoted by the symbol T. The relationship between the shear stress and shear rate is used to define the flow properties of fluid foods.

Newton's law states that for flowing fluids, the shear stress required to maintain the flow is proportional to the shear rate.

i.e. $$F/A = \mu \frac{du}{dx}$$

Where du/dx = velocity gradient (shear rate) and μ is called the viscosity (poise) of the fluid. This is equal to:

$$\mu = \frac{F}{A} \times \frac{dx}{du}$$

$$= T \cdot \frac{|}{\gamma}$$

$$\mu = T / \gamma$$

where T = Shear stress
γ = Shear rate

where γ is the shear rate.

The unit of viscosity (poise) is dynes.sec/cm^2. In SI units it is equal to 0.1 N s/m^2 where N is Newton. As this unit is large, usually centipoise (cp), which is 1/100 poise, is used.

Fluids having this type of flow behaviour are called Newtonian fluids. Several fluid foods like fruit pulps, fruit juice concentrates, ketchup and sauces do not obey this law. Therefore, such fluids are called non-Newtonian fluids. For such fluids, if measurements are made at different shear rates, the ratio of shear stress to shear rate will not be constant. This ratio is called the apparent

viscosity (μ_a or μ_{app}) or consistency. The consistency of non-Newtonian fluids can be expressed by the power law equation:

$$\tau = K (\gamma)^n + C$$

where, K = fluid consistency coefficient (dynes^n sec cm^2)
n = flow behaviour index (non dimensional)
C = yield stress (dynes / cm^2)

It can be seen from the equation that in the case of Newtonian liquids, n is equal to one and K becomes the viscosity. The constant, n for non-Newtonian liquids is a measure of the extent of departure from Newtonian behaviour, and hence is called the **flow behaviour index**. The constant K is more a measure of viscosity or consistency and is termed the fluid consistency coefficient. The viscometric parameters of non-Newtonian fluids can be determined with either capillary tube or rotational viscometers. Since determination of the parameters is very elaborate and not required for routine quality control of products, the same is not discussed here. Instead, a few viscometers and consistometers commonly used are described here.

4.3.2 Bostwick Consistometer

The Bostwick consistometer is a simple devise widely used in the industry for measuring the consistency of tomato ketchup and sauce. The instrument is based on the principle that the length of flow of the sample is proportional to its consistency.

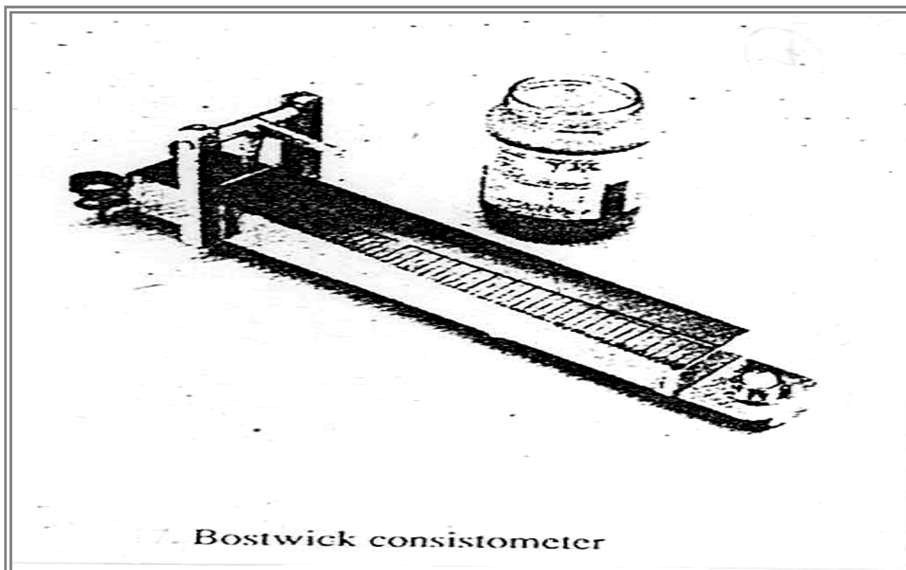

Figure 4.7: Bostwick consistometer

The Bostwick consistometer (Figure above) consists of a channel with sides. It has a triggered gate on one side and a centimetre scale is itched on the floor of the channel. Before taking measurements, the consistometer is levelled by adjusting the levelling screws provided at the bottom of the instrument. Then the gate is closed by engaging the trigger release mechanism and the sample is filled in the sample holding compartment fully. The gate is released by the trigger and simultaneously a stopwatch is started. The consistency is measured after 30 sec by recording the extent of flow of the sample on calibrated scale,

taking an average of the values at the centre and sides of the scale. The Bostwick consistometer readings are expressed as cm per a fixed time.

4.3.3 Brookfield Synchrolectric Viscometer

The Brookfield synchrolectric viscometer is a versatile instrument that can be used for measuring viscosity of Newtonian liquids as well as the consistency of non-Newtonian liquids. The instrument is based on measurement of resistance to rotation of a spindle immersed in the test material. The resistance is recorded in terms of torque by a calibrated spring. The dial of the instrument is graduated and the viscosity in centipoises can be read directly from the factor finder for different sizes of spindles supplied along with the instrument. As the instrument is supplied with different sizes of spindles, a wide range of viscosity measurements can be made.

The instrument is provided with a high torque motor, which is geared for different speed. Therefore, measurements can be made at different shear rates, which is required for non-Newtonian liquids. Brookfield viscometer is widely used to measure the consistency of products like tomato products, custards, dairy products, cream style corn etc.

4.4 TEXTURE

Texture is the property of food, which is associated with the sense of feel or touch experienced by fingers or the mouth. Texture of foods perceived by the mouth is a very complex phenomenon. It is perceived in three stages of ingestion of food viz. initial, masticatory and residual each consisting of different textural parameters. Therefore, objective quantitative description of the texture perceptions is quite difficult.

The physical or the mechanical textural characteristics of foods are related to the reaction of the food to stress and can be divided into parameters of hardness, brittleness, chewiness, gumminess, cohesiveness, viscosity, elasticity and adhesiveness.

In objective measurement of texture, food is subjected to compression, tension, shear and flow and the resultant deformation is measured. Measurements are made in terms of integral powers of force (m), length (l) and time (t).

All texture measuring instruments/ devices have a few essential parts. They are: I) driving mechanism to apply force, ii) probe element in contact with the sample, iii) mechanism to suitably direct the applied force, iv) sensing element, and v) read out system.

The instrumental methods of measuring the texture are based on applying force under controlled conditions. Standardisation of test cells for different types of products is crucial for getting useful data, which can be interpreted to give meaningful results. Each product in each test cell produces a characteristic force-deformation curve. Under controlled conditions of the test, the magnitude of the curve is influenced by the textural behaviour of the material tested. A typical force- deformation curve is shown in the following Figure.

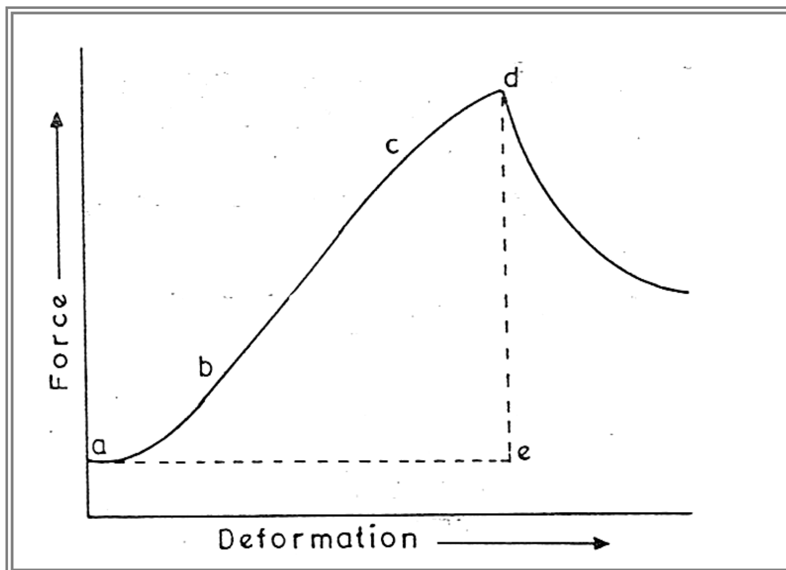

Figure 4.8

In general, the curve exhibits the following characteristics.

i) An initial non-linear portion representing packing of the cellular components of the sample under the applied stress.

ii) An approximately linear portion as the material is compressed. This slope of this portion of the curve represents the force required to attain a given deformation and hence a measure of the firmness.

iii) An abrupt change in the slope is seen when the sample begins to rupture.

iv) After the rupture point d, the force reduces.

There are different types of texture measuring instruments. Some instruments are very simple, measuring only a single mechanical textural characteristic of the product. Quite often, this is sufficient for quality control purposes. Fruit pressure tester like the Magness-Taylor pressure tester is an example of such an instrument. However, for research and development, one would like to get more extensive information on the various textural parameters of the product. This is called **texture profile analysis**.

4.4.1 Magness-Taylor Pressure Tester

This hand held instrument is widely used to determine the softening of fruits during maturation. The pressure tester uses a spring to measure applied force and a spring scale indicates the maximum test force. The tester consists of a metallic barrel inside which a spring is placed. A shaft to which a removable punch (plunger) is attached supports the spring (Figure 4.9). A small chuck at the end of the shaft ensures the fixed length of penetration. The instruments are available with springs of different force ranges to suit objects of different firmness. Each instrument is provided with punches of different diameters. The smaller diameter punches are used on firmer material and the larger on softer material.

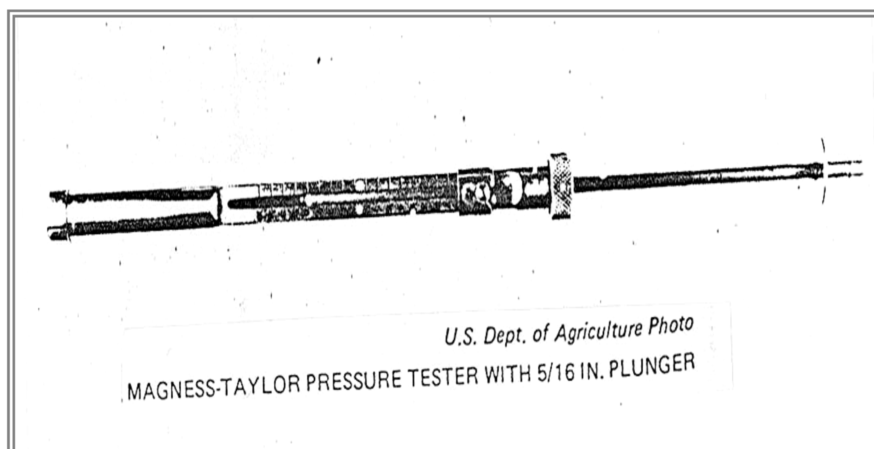

Figure 4.9

For measuring the pressure of a fruit, the plunger is held against the surface of the fruit and forced into the fruit with steady pressure applied by the hand to attain the necessary force necessary for breaking the flesh. The fruit may be peeled to overcome the interference of the skin with the action of the plunger. The force recorded on the scale indicates the maturity of the fruit. Typical range of pressure values of one variety of apples at different maturity stages is given below:

Degree of maturity/ripeness	Pressure test reading (lb) Delicious apple
Hard	17-20
Firm	14-17
Firm-ripe	11-14
Ripe	8-11

4.4.2 Instron Testing Machine

This is a versatile instrument capable of obtaining texture profiles of different types of objects. The machine consists of two parts: i) the drive mechanism, which drives a moving cross head in a vertical direction in selected speeds of 0.05 to 50 cm / min and ii) the load sensing and recording system for loads in the range of 2g to 5,000 Kg. There are different types of fixtures like flat compression plates, cylindrical compression box, assortment of needles and punches, a single star-shaped needle etc. A careful selection of the fixtures permits the measurement of different texture parameters. The instrument measures force- deformation of force- time functions. It is extensively used to measure the texture of fruits, vegetables and processed food products.

4.4.3 Measurement of Jellying Property of Pectins

Jams, jellies and marmalades are important commercial products. Pectin is commonly used to obtain the characteristic jelly like texture to the products. Therefore, the jellying quality of the pectin used determines the product quality. There are different methods for assessing the quality of pectin. Physical methods for determining jelly strength are two types. The first group of methods measure the breaking strength of jellies when they rupture after exceeding their elastic limits and the second group of methods measure jelly

strength by taking into account the deformation of jellies within their elastic limits. Here one method in each group is described.

Pectinometer

The Luers-Lochmuller Pectinometer (Figure 4.10) measures the force required to pull a disc embedded in the jelly being tested upwards. The apparatus is shown below. It consists of a moving load (weight) put on one arm of a balance and the disc in the jelly attached to the other arm through a double pulley arrangement. The jelly container (corrugated) is held in a fixed position on the base of the balance (B). The disc (A) is suspended in the corrugated container (C). The jelly being tested is poured hot into the container and allowed to cool for at least one hour before the test is carried out. Now, weights are applied on the pan (W) till the jelly breaks. The corrugated sides of the container prevent slipping of the jelly in the container. The indicator (I) measures the extent of compression of the jelly. Breaking values pf jellies range from 200 to 300 g depending on the type of jelly. For quality control purposes a narrow range (± 15g) is usually fixed.

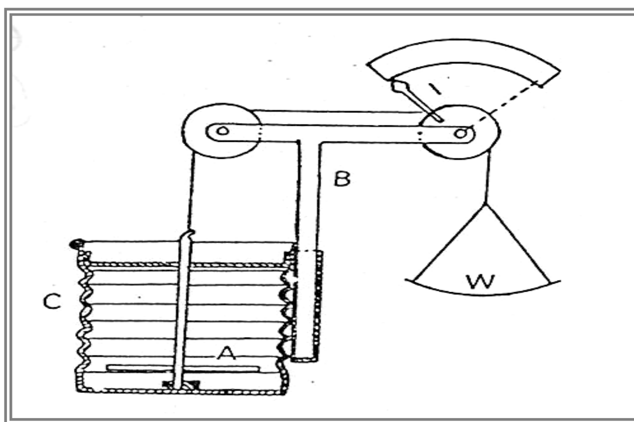

Figure 4.10: Luers and Lochmuller pectinometer

Ridgelimeter (Cox-Higby Sag Method)

Ridgelimeter measures the percentage sag or slump occurring when a test jelly is removed from its supporting container and inverted upon a glass plate. The Fig. below shows the instrument with a sample of jelly in measuring position and jelly glasses (containers). The glasses are of standard sizes of 3.125 in (79.4 mm) depth. The instrument as such has only a micrometer screw fixed on a stand. The screw has 32 threads to an inch so that one revolution moves the point by 0.03125 in., which is equal to 1% of the height of the jelly. Therefore, if the depression of the jelly as measured by the instrument is 0.03125 in. it is equal to 1% sag. For determining the jelly strength of a pectin sample, the following procedure is followed.

Weigh 48.8 g of tartaric acid in distilled water and make up to 100 ml in a volumetric flask. The quantity of pectin to be taken for the test depends on the assumed grade of the pectin. For example commercial pectin is usually supplied as 150 grade. Therefore, the assumed grade of the pectin is taken as 150. Since jellies should contain 65% sugar (TSS) i.e. 65 g sugar in 1000 g jelly, the weight of pectin to be used is 650/assumed grade of the pectin (650/150 = 4.33 g). Weigh 646 g sugar and 4.33 g pectin. Mix the weighed pectin with about 20-30 g of the weighed sugar in a dry beaker. The tare weight of a stainless steel saucepan along with a stirrer (about 1 lit. cap.) is

noted and 410 ml of distilled water is taken in the pan. The pectin-sugar mix is added to the water in the pan and stirred. The pan is placed on a hot plate and heated to boil the solution. The remaining sugar is added and heating and stirring continued till the sugar dissolves completely. Heating is continued until the net weight of jelly is 1015 g. If the net weight is less, distilled water is added in slight excess and boiled down to exact weight. The entire heating time should not exceed 5-8 min. The material is allowed to stand for 1 min., any foam or scum is skimmed off and allowed to cool to 95°C (check with a thermometer) while stirring gently. Pour the hot jelly into three Ridgelimeter glasses (after fixing gummed tapes to a height of 0.5 in. above the rims) almost to overflowing, each containing 2 ml of the tartaric acid solution. After 15 min., the glasses are covered with metal lids and allowed to cool for 20-24 hr. at 25° ± 3°C.

After cooling, the lids are removed and the gummed tapes are tore off. The jelly exposing beyond the brims of the glasses is sliced off with a stretched wire. Now the jelly from one glass is removed with the help of a spatula and directly placed on to the glass plate provided along with the instrument. Start a stopwatch as the jelly is placed on the glass plate, and place the jelly directly below the micrometer screw. After exactly 2 min. bring the point of the micrometer screw just into contact with the jelly surface. Read the sag to the nearest 0.1%. Repeat the measurements with the jellies in the other two glasses also and take the average. The true grade of the pectin is calculated by multiplying the assumed grade with the corresponding factor for the measured sag provided in a tabular form along with the instrument. For example if the assumed grade is 150 and the factor for the measured sag of say 25 is 0.936, the true grade of the pectin is:

$$150 \times 0.936 = 140.4 \text{ or } 140 \text{ grade}$$

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. State Newton's law and differentiate between viscosity and consistency?

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2. Describe the use of Bostwick consistometer.

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3. What is meant by the texture of a material?

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4. Describe a simple instrument to measure the pressure of a fruit.

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5. Which are the essential components of the Instron Testing machine? What is its speciality?

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6. How the Ridgelimeter is used to measure jelly strength?

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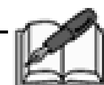
4.5 LET US SUM UP



Measurement of colour, viscosity and texture of foods is very important for quality control.

When visible white light falls on an object, some of the spectral colours of the light are partially or fully absorbed by the object. The resultant coloured radiation is reflected from a solid or semi-solid object or transmitted through a liquid object, which the eyes perceive as the colour of the object. Any colour can be matched by mixing the three primary colours viz. red, green and blue in appropriate proportions. The relative amounts of the three primary colours required to match a colour is called the Tristimulus value of the colour. This is the basis of quantitative specification of colours under the CIE system. The CIE system specifies a colour in terms of its Hue or dominant wavelength, Chroma and Lightness. Based on instrumental measurement of reflectance from an object over the entire light spectrum, it is possible to arrive at the complete specification of its colour. For routine quality control of some food products like fruits, vegetables, meat etc., simple comparison of their colour with standard colour charts gives useful data. The Hunter colour system which is related to the CIE system specifies a colour in terms of L, a, b values.

Foods can exist in the liquid, semi-solid and solid state. Liquid foods are classified as Newtonian or non-Newtonian liquids depending on their flow properties. Instruments are available to measure the flow properties of liquid foods.

Texture of solid foods is related to their reaction to stress and can be divided into parameters of hardness, brittleness, chewiness, elasticity etc. Texture of foods is determined by measuring the relationship between the applied pressure and the resultant deformation of the food material. There are simple instruments like the fruit pressure tester, which measure a single parameter of texture like the pressure required for puncturing a fruit. Such data are usually sufficient for some quality control purposes. However, more detailed texture analysis is required for many other products especially for research and product development. Such texture profile analysis is possible with instruments like the Instron Testing Machine.

Jams, jellies and marmalades are important commercial products. Quality control of such products require measurement of their jell quality. The gel quality is mainly determined by the quality of the pectin used for their preparation. The jell strength of pectin and the products are determined using simple instruments. The instruments are either based on measuring the force required to break a gel or the sag (loss of rigidity) of a gel on standing.

4.6 KEY WORDS

Hue : The colour attribute denoted by red, green, blue and so on.

Saturation : The depth or strength of hue.

Lightness : The brightness of colour.

Dominant wavelength : Wavelength of the spectrum light that, when combined in suitable proportions with the specified achromatic (colourless) light yields a match with the light considered.

Tristimulus values : The amounts of the three primary colours required to match a colour.

Rheology : Study of flow and deformation of materials.

Newtonian fluids : Fluids for which the shear stress required to maintain its flow is proportional to the shear rate.

Texture profile analysis : Determination of various texture parameters like hardness, cohesiveness, elasticity, adhesiveness, fracturability, gumminess, chewiness etc.

4.7 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   - Appearance property
   - Absorption of some spectral wavelengths
   - Reflection and transmission of light

2. Your answer should include the following points:

   - Hue
   - Saturation
   - Lightness

3. Your answer should include the following points:

   - Primary colours
   - Tristimulus values
   - Chromaticity coordinates
   - Chromaticity chart
   - Dominant wavelength
   - Standard illuminants
   - Standard observer

4. Your answer should include the following points:

   - L,a,b values
   - Colour difference

Check Your Progress Exercise 2

1. Your answer should include the following points:

   - Ratio of shear stress to shear rate constant
   - Power law equation
   - Flow behaviour index

2. Your answer should include the following points:

   - Flow meter
   - Flow per unit time

3. Your answer should include the following points:

   - Deformation due to application of force
   - Hardness
   - Cohesiveness
   - Elasticity
   - Fracturability
   - Gumminess
   - Chewiness

4. Your answer should include the following points:

   - Magness-Taylor Pressure tester
   - Force required for puncturing

5. Your answer should include the following points:

   - Drive mechanism
   - Load sensing and recording system
   - Texture profile

6. Your answer should include the following points:

   - Standard jelly
   - Measurement of per cent sag

4.8 SOME USEFUL BOOKS

1. Kramer, A. and Twigg, B.A. (1966) Fundamentals of quality Control for the Food Industry, The AVI Publishing Co., Inc., Westport.

2. Hutchings, J.B. (1994) Food Colour and Appearance, Blackie Academic & Professional, London.

3. Owen R. Fennema (1976) Principles of food science, Part II-Physical Principles, Marcel Decker Inc.; New York.

4. Ranganna, S. (2000) Handbook of Analysis and Quality Control for Fruit and Vegetable Products, Tata McGraw-Hill Publishing Co., Ltd., New Delhi.

UNIT 5 TESTING AND EVALUATION: CHEMICAL AND MICROBIOLOGICAL

Structure

5.0 Objectives
5.1 Introduction
5.2 Chemical Analysis of Foods
Crude Fat or Ether Extractives
Protein Estimation
Pectin Estimation
Estimation of Tannins
5.3 Bacteriological Examination of Water
Plate Count
Coliform Count
Faecal Streptococci Test
5.4 Assessment of Surface Sanitation
5.5 Microbiological Examination of Food Spoilage
5.6 Let Us Sum Up
5.7 Key Words
5.8 Answers to Check Your Progress Exercises
5.9 Some Useful Books

5.0 OBJECTIVES

After reading this unit, you should be able to:

- describe the methods for determining crude fat, protein, pectin and tannins in food products;
- discuss various aspects of bacteriological examination of water;
- describe procedures for assessing surface sanitation; and
- explain the salient aspects of microbiological examination of spoiled canned foods.

5.1 INTRODUCTION

Analytical food chemistry deals with the methods for determining the chemical composition (quality) of foods. It employs both qualitative and quantitative methods for the purpose. A qualitative method yields information about the nature of the component and a quantitative method provides numerical information on the content of the component in the sample.

For the analysis of foods, chemical methods are more often employed even though physical methods are also proving to be very useful. The analytical methods based on the physical properties of food components are collectively known as “Instrumental Methods of Analysis’, which you will be learning in the next Block.

You will be learning and performing several analytical methods in your Practical exercises under Courses III and VII. In this unit, you will learn a few other methods, which are, either too time consuming or could not be included under the Practical exercises because they require more sophisticated facilities which are available only in specialised laboratories.

Microbiological quality of foods is equally important. A major portion of microbiological methods will be covered under Course V 'Food Microbiology". Therefore, in this Unit, only a selected few methods are discussed.

5.2 CHEMICAL ANALYSIS OF FOODS

The quantitative analysis of food raw materials and their products may be classified into **proximate analysis** and **ultimate analysis**. Proximate analysis provides information on the nutritional and biochemical composition, while ultimate analysis or detailed analysis determines the content of a particular component in the food material.

The proximate analysis, especially for fruits and vegetables consists in determining the percentages of the moisture, ash, acidity, crude fat or ether extractives, protein, sugars and crude fibre. Their sum total subtracted from 100 represents primarily the amount of carbohydrates other than sugars, but includes starch, pectin, etc.

Among the above constituents, all except **crude fat, protein** and **pectin** are covered in the Practical exercises. Therefore, these methods are described in this unit. Tannin, which is another component of importance to fruits and vegetables, is also included here.

5.2.1 Crude Fat or Ether Extractives

As you have learnt already, food products contain water-soluble and water insoluble constituents. Among the water insoluble constituents, the ether soluble materials such as the tri- glycerides, phospholipids, sterols, essential oils, fat-soluble vitamins and pigments are very important. The first step in determining most of the individual components is extracting the food material with ether using a Soxhlet extraction apparatus (Figure 5.1). The Soxhlet extraction apparatus is an all glass simple assembly consisting of a Soxhlet flask to the top of which a Soxhlet extraction tube and a condenser are attached through standard glass joints. The Soxhlet extraction tube has provision for siphoning off the solvent used for the extraction when the level has reached a particular level.

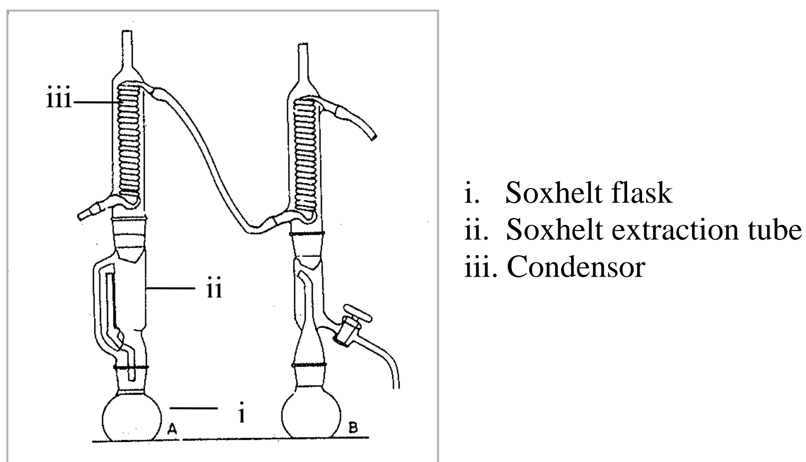


Figure 5.1: Soxhlet apparatus (A) for fat extraction and (B) for solvent removal

Ether extractives are determined on samples after moisture determination (please see 'Moisture determination' under Course III Practical exercises). The dried sample is transferred to a thimble. The thimble has a tubular structure like a large test tube (having diameter and length suitable to occupy

the Soxhlet extraction tube) made of cellulose filter pad. The top of the thimble is plugged with a wad of pure cotton and placed inside the Soxhlet extraction tube, which is then attached to the Soxhlet flask. About 75 ml of anhydrous ether is poured through the sample in the thimble, which percolates through the sample into the flask. The condenser is then attached and the whole assembly is placed in a heating mantle or water bath. Heat is applied just enough to boil the ether. The evaporated ether condenses and falls into the thimble containing the sample and extracts the ether soluble components. When the ether level reaches the siphon outlet level, it is siphoned off into the flask. The distillation process is allowed to continue for about 16 hours. At the end of the extraction period heating is discontinued and the thimble is removed. The ether-extracted sample is used for crude fibre estimation (please refer Practical exercise under Course III). After removing the thimble, ether is distilled off into the Soxhlet tube and poured out before the level reaches the siphoning level. Ether may be distilled off using the assembly B shown in the Fig. When the ether in the flask has reached a small volume, it is transferred into a weighed beaker with repeated rinsing with small portions of ether. The ether in the beaker is evaporated on a steam bath, dry at 100 ^oC for 1 hr, cooled and weighed. Crude fat is calculated by:

$$\% \text{ Crude fat} = \frac{\text{Weight of ether-soluble material}}{\text{Weight of sample}} \times 100$$

5.2.2 Protein Estimation

Protein content of food samples is usually determined by the Kjeldahl method, which is based on the determination of the amount of reduced nitrogen present in the sample. The nitrogen compounds are converted into ammonium sulphate by boiling with concentrated sulphuric acid. The ammonium sulphate formed is decomposed with sodium hydroxide and the liberated ammonia is absorbed in excess of neutral boric acid solution and titrated with standard acid.

The food sample is digested (destruction of organic matter) with conc. H_2SO_4 in a Kjeldahl flask that is a long neck round bottomed flask. To facilitate digestion and efficient conversion of all the reduced nitrogen into ammonium sulphate, small quantity of a catalyst is added. The catalyst consists of a mixture of selenium dioxide, potassium sulphate and copper sulphate in the ratio of 1:40:8. The digested and cooled sample is made up to a known volume with distilled water and an aliquot of it is distilled in a micro-Kjeldahl distillation apparatus (Figure 5.2).

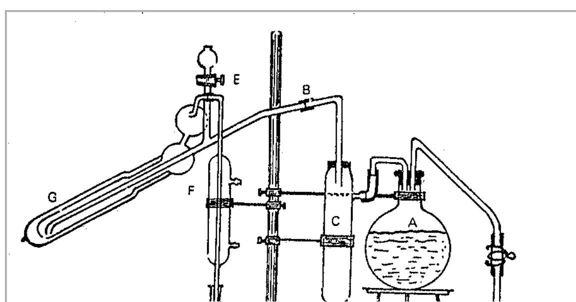
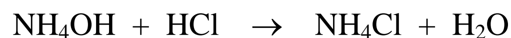


Figure 5.2: Distillation apparatus for micro-Kjeldahl determination of nitrogen *A* steam generator, *B r*ubber tubing, *C* steam trap, *D* pinch clamp, *E* funnel, *F* condenser, *G* distillation flask

This is a glass unit with only one short rubber tube joint (B). This rubber joint makes the unit less rigid and reduce the danger of breakage. The steam generator A is a 1-litre round-bottomed flask with a side arm for refilling. The other components of the assembly are the steam trap C, sample delivery funnel E, condenser F and sample holder for steam distillation G.

After initial cleaning of the system with steam, an aliquot of the acid digested sample and strong sodium hydroxide solution are let into the distillation flask and steam distilled. The liberated ammonia is absorbed in a known volume of dilute neutral boric acid solution containing an indicator (mixed indicator) placed below the condenser. On completion of distillation, which takes about 5 min the ammonia absorbed boric acid solution is titrated with 0.01 N HCl. From the titre value, the nitrogen content in the sample is calculated. The function of boric acid in the estimation is only to absorb the liberated ammonia and does not react chemically with it. The titration reaction is:



$$NH_4OH + HCl \rightarrow NH_4Cl + H_2O$$

Calculations:

1000 ml 1 N HCl = 1 g mole of nitrogen = 14 g nitrogen

or 1 ml 1 N HCl = 14 mg nitrogen

Therefore

$$\% \text{ N in the sample} = \frac{\text{Titre x normality of HCl} \times 14 \times 100}{\text{Aliquot of digest taken} \times \text{weight of sample} \times 1000}$$

For calculation of protein content from the nitrogen value, a multiplication factor of 6.25 is usually used. This factor is based on the assumption that plant proteins contain 16 % nitrogen. Therefore, protein content (%) = % N × 6.25.

5.2.3 Pectin Estimation – Calcium Pectate Method

Pectin is an important constituent of fruits. Processing wastes of fruits like citrus peel and pomace, apple pomace etc. are used for commercial, production of pectin. Knowledge of the pectin content of fruits used in jam and jelly manufacture is necessary to calculate the quantity of pectin to be added. Therefore, estimation of pectin content of the raw materials is important.

Pectin extracted from plant materials is usually estimated as calcium pectate. For the estimation, the pectin sample is saponified with alkali and precipitated as calcium pectate from an acid solution by the addition of calcium chloride. The calcium pectate is washed free of chloride, dries and gravimetrically estimated.

Pectin extraction from plant materials: 50 g sample is weighed into a 1000 ml beaker and extracted with 400 ml 0.05 N HCl for 2 hr at 80 – 90°C. Water lost by evaporation is frequently replaced. After cooling the suspension, it is transferred to a 500 ml volumetric flask and made up to volume with distilled water. Subsequently, it is filtered through Whatman No. 4 filter paper.

100-200 ml of the filtrate is taken in a 1 lit beaker and 250 ml distilled water is added to it. The solution is neutralized with 1 N NaOH using phenolphthalein as the indicator and allowed to stand overnight. The next day, 50 ml 1 N acetic acid is added to the solution, stirred and after 5 min, 25 ml 1 N calcium chloride solution is added with stirring. After 1 hr, the solution is boiled for 1-2 min and filtered through a previously dried and weighed (in a covered dish) filter paper circle. The precipitate in the filter paper is washed with almost boiling water till free of chloride. Presence of chloride in the filtrate is tested with silver nitrate solution. The filter paper containing calcium pectate is transferred to the covered weighing dish initially used, dried overnight at 100°C, cooled in a desiccator and weighed. Calcium pectate is calculated as follows.

$$\%\ \text{Calcium pectate} = \frac{\text{Weight of calcium pectate} \times 500 \times 100}{\text{ml of pectin extract taken} \times \text{Wt. Of the sample}}$$

The theoretical yield of calcium pectate from pure pectinic acid is about 110%.

5.2.4 Estimation of Tannins

Tannins are widely distributed in fruits and vegetables. They belong to the general group of polyphenols called flavonoids and cinnamic acid derivatives. Tannins are responsible for the undesirable astringency of cashew apple or desirable astringency of apple and apple juice, amla etc. They are responsible for the typical taste and aroma of tea and cocoa. Polyphenols play important role in protecting ascorbic acid in some fruits and can act as a anti-oxidants. Of late, polyphenols have been shown to have several health benefits including prevention of diseases like heart attack, cancer etc.

Tannins can be estimated either by volumetric or by colorimetric method.

Volumetric method

The volumetric method of estimation of tannins is based on the oxidation of tannins by potassium permanganate.

Reagents

1. 0.04 N Potassium permanganate solution (1.40424 g $KMnO_4$ per litre).
2. Indigo carmine solution: 1.5 g indigo carmine in 1 litre water containing 50 ml conc. H_2SO_4.
3. Gelatin solution: Soak 25 g gelatin in saturated sodium chloride solution for 1 hr, heat to dissolve, cool and make up to 1 litre with saturated sodium chloride solution.
4. Acid sodium chloride solution: To 975 ml saturated sodium chloride solution, add 25 ml conc. H_2SO_4.

Procedure

An aliquot of the filtered fruit juice or extract (10 to 20 ml containing about 0.01 g of tannin) is taken in a porcelain dish and 20 ml of the indigo carmine solution is added followed by 500 ml water. From a burette the potassium permanganate solution is added to the dish with vigorous stirring until the colour becomes light green. Then the permanganate solution is added drop wise till the colour changes to bright yellow. The titre value is noted as *A*. Now, another aliquot of the juice or fruit extract (50 ml equal to 10 ml of original juice or extract) is taken in a 250 ml volumetric flask and 25 ml of the gelatin solution is added and made up to volume with the acid sodium chloride solution. It is transferred to a conical flask, a little of filter aid is added, shaken well and filtered. To 50 ml of this filtrate, 20 ml indigo carmine solution is added and titrated with the permanganate solution as done previously. The titre value is noted as *B*.

Calculations

A = Total tannin like materials

B = Non tannin materials

A – *B* = True tannins

One ml of 0.04 N $KMnO_4$ = 0.00168 g tannin (Gallo tannic acid)

$$\% \text{ Tannin} = \frac{(A - B) \times 0.00168 \times 100}{\text{Volume of sample}}$$

Colorimetric method

The colorimetric method for estimation is based on the measurement of blue colour formed by the reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline medium. The following reagents are required for the estimation.

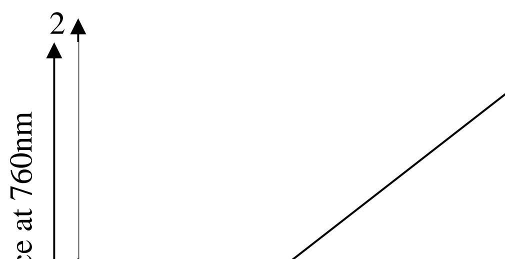
i) Folin – Dennis reagent: A mixture of 100 g of sodium tungstate ($Na_2WO_4.2H_2O$), 750 ml water and50 ml 85% phosphoric acid (H_3PO_4) is refluxed for 2 hr, cooled and diluted to 1000 ml with water.

ii) Saturated sodium carbonate solution: 35 g anhydrous sodium carbonate is dissolved in 100 ml warm (70-80°C), and cooled. The clear supernatant solution is used for the estimation.

iii) Tannic acid standard solution: Dissolve 100 mg tannic acid in 1 litre water (1 ml = 0.1 mg tannic acid)

Standard curve

To a series of 100 ml volumetric flasks containing 75 ml water, 0 to 10 ml aliquots of the standard tannic acid solution is pipetted followed by 5 ml Folin-Dennis reagent and 10 ml saturated sodium carbonate solution. Make up to volume with water, mix and measure the absorbance (colour) of the solutions after 30 min in a colorimeter or spectrophotometer at 760 nm. A standard curve is prepared by plotting mg tannin in the x axis and the corresponding absorbance on the y axis.



Mg of tanin

Tannin estimation in sample

Liquid samples can be used as such. Solid samples (about 5 g) are boiled with water for 30 min, cooled and made up to a known volume.

An aliquot of the prepared sample (containing about 0.1 mg tannin) is used for colour development as in the case of standard. Note the tannin content from the standard curve and the tannin content in the sample is calculated.

Calculations

Weight of sample taken = W ------- g

Sample extract made up to = V ------- ml

Volume taken for colour development = V_1 ------- ml

Tannin content in V_1 ml extract (from standard curve) = a ------- mg

$$\text{Tannin as \% tannic acid} = \frac{a \times V \times 100}{V_1 \times W \times 1000}$$

If liquid sample is taken for estimation as such:

$$\text{Tannin as \% tannic acid} = \frac{\text{mg tannic acid in sample aliquot} \times 100}{\text{ml sample aliquot take} \times 1000}$$

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Differentiate between Proximate analysis and ultimate analysis.

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2. Describe the method for estimating the crude fat content of a food product.

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3. Describe the micro-Kjeldahl method for determination of nitrogen. How the nitrogen value is converted to protein?

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4. Describe the calcium pectate method for pectin estimation

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5. What is the importance of poly phenolic compounds in foods? Explain the volumetric method for tannin estimation.

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5.3 BACTERIOLOGICAL EXAMINATION OF WATER

Large quantities of water are used in the food processing industries for various purposes. Some of the chemical qualities of the water are discussed in another course. Bacteriological quality of water is equally or more important from the public health point of view. The main objectives of bacteriological examination of water required for the food processing industry are to check whether:

i) the water is safe for human consumption,
ii) the water requires treatment,
iii) the treatment followed is adequate.

The following parameters are usually included in the bacteriological examination of water:

i) plate count,
ii) coliform count,
iii) faecal Streptococci test.

Sampling of water: Care should be taken to collect samples of water for bacteriological examination of water. Water should be collected fresh in sterilizes glass bottles and closed properly.

5.3.1 Plate Count

Plate count or total plate count is determined by mixing a known volume of the water sample with a solidifiable culture medium and incubating it for a specified time to allow formation of visible colonies.

Requirements

i) Nutrient agar sterilized in 15 ml quantities in plugged culture tubes.

ii) Sterilized Petri dishes of 10 cm diameter.

iii) Ringer's solution (full strength): Dissolve 9 g sodium chloride, 0.42 g potassium chloride, 0.48 g calcium chloride and 0.2 g sodium bicarbonate in 1000 ml distilled water. Suitably diluted solution is sterilized at 15 psig for 20 min before use.

iv) Dilution bottle each containing 90 ml of quarter-strength Ringer's solution.

v) Sterilized 1 ml and 10 ml pipettes.

Procedure

You have already learnt sample dilution, plating etc. The agar plates are incubated at 37°C for 24 ± 3 hr. After incubation, the colonies formed are counted using a colony counter. The results are expressed as **"plate count per ml at 37°C in 24 hr."** Properly filtered and chlorinated water will not have total plate count in excess of 10 per ml.

5.3.2 Coliform Count

As you have learnt already, presence of coliforms in water indicates the possibility of faecal contamination and hence is strictly monitored. Coliform counts involve three tests viz. i) presumptive test, ii) confirmed test and iii) completed test.

Presumptive test: E-coli is one of the few bacteria, which is able to ferment lactose (lactose broth) with production of acid gas. Therefore, a positive test indicates 'presumptive' evidence for sewage contamination in the water sample. It is only 'presumptive' because several non-faecal bacteria also answer this test. Hence, this test must be 'confirmed'.

Confirmed test: Positive 'presumptive' test sample is plated on eosin-methylene blue (EMB) agar and incubated at 37°C for 24 hr and examined. Typical E-coli colonies will have dark to black centres, button-like in appearance and will often be surrounded by a greenish metallic shine. If positive 'presumptive' sample inoculated into brilliant green lactose bile broth and incubated at 37°C for 24 hr shows gas formation also, it is confirmatory evidence for the presence of E-coli.

Completed test: Completed test for E-coli is performed by transferring a typical colony from EMB agar plate to a nutrient agar slant and lactose broth tube and incubating for 24hr at 37°C. A stained mount of the agar culture is examined under a microscope. A completed test for E-coli should show a pure culture of Gram-negative short rods, and gas formation in the lactose broth tube.

5.3.3 Faecal Streptococci Test

The genus Streptococcus constitutes a diverse group of cocci widely distributed in nature. Some are dangerous pathogens. Streptococci found in faeces are called faecal Streptococci or Enterococci of which Streptococcus faecalis is typical. Streptococci usually occur in pairs of ovoid cocci or in short chains. Unlike many other Streptococci, these grow well in ordinary laboratory media in the presence of bile salt. They produce deep red pin pointed colonies in MacConkey agar and ferment glucose, lactose and mannitol, producing acid but no gas. While most Streptococci are susceptible to heat (55° C for 15 – 20 min), Streptococcus faecalis offers relatively high resistance destructive agents such as heat and can withstand a temperature of 60° C for 30 min. In examining water for the presence of faecal Streptococci, advantage is taken of its relative heat resistance, its ability to form acid in MacConkey broth, and grow in the presence of a concentration of sodium azide sufficient to prove inhibitory to most coliform bacteria. As some anaerobic spore forming bacilli also produce acidity in sodium azide medium, a confirmatory test is also performed. In the confirmatory test, a heavy inoculum from the positive azide tubes is plated on MacConkey agar and incubated at 37° C for 48 hr. The growth of minute pin pointed red colonies is a strong evidence of the presence of faecal Streptococci. On Gram-staining and observing under the microscope, the Streptococci appear as Gram-positive.

5.4 ASSESSMENT OF SURFACE SANITATION

Bacterial counts on utensils, equipment, working surfaces, walls, floors etc., are useful means of assessing the standard of hygiene and the efficiency of cleaning procedures in food factories. The **'Swab Rinse Method'** is useful for the purpose.

For the swab method, pre-sterilized cotton swabs are used. The sterile swabs are dipped in sterile Ringer solution and rubbed over the required surfaces. The rubbed swabs are allowed to stand in sterile Ringer solution for 20-30 min and shaken. This solution is used to make plate counts with yeast extract agar. The Ringer solution inoculated into MacConkey broth should not show

positive test for coliform organisms for well-sanitised surfaces. For bins and large utensils, sterile Ringer solution is used to rinse them. The rinsed solution is plated and inoculated in MacConkey broth as above.

5.5 MICROBIOLOGICAL EXAMINATION OF FOOD SPOILAGE

Microbiological spoilage of foods and its examination is an extremely wide area. Therefore, in this section, microbiological examination of spoilage of canned vegetable products, which is the most important, is discussed.

Canned Products

Canned food spoilage has both monitory loss and public health implications. Microbiological spoilage of canned fruits and vegetables is due to either under processing or post processing contamination (leakage). Under processing is due to insufficient heat treatment resulting in failure to destroy all microorganisms capable of subsequent growth in the product. Leakage is due to contamination of the product, mostly during the cooling process due to faulty seam or damage to the can. Microbiological examination of the spoiled cans help in identifying the cause of spoilage and take remedial measures.

The nature microorganisms associated with spoilage of canned fruits and vegetables is related to the pH of the products. As you have learnt, the spoilage of acidic foods like most fruit products is less critical from safety point of view. The pH of most canned fruits ranges from 3.7 to 4.5. In such products, spoilage is usually caused by aerobic and anaerobic spore formers, though Lactobacilli and Leuconostocs are also encountered occasionally. Tomato is a critical product having pH in the borderline between acidic and low acid foods. Butyric anaerobes and aciduric flat sours are important spoilage organisms of tomato products.

Spoilage in low acid (pH more than 4.5) canned products like vegetables; vegetable soups etc., spoilage due to under processing are caused by thermophiles. Some of these organisms and the type of spoilage are given below.

Bacillus stearothermophilus	:	Causes flat sour spoilage
Cl. thermosaccharolyticum	:	Causes hard swell
Cl. nigrificans	:	Causes sulphur stinkers
Mesophilic spore formers	:	Cause putrefaction

Spoilage due to can leakage is characterised by the presence of a variety of organisms including non-thermophilic organisms like various cocci including Leuconostoc and micrococci. The presence of micrococci and/ or yeasts is almost certain evidence of can leakage.

Examination of can spoilage is an elaborate process. The first step is sampling. If the spoilage is widespread, about 6-12 cans from each batch may be sufficient. Other wise the number of cans required will be quite large. The sampled cans are subjected to the following examinations.

Physical examination: The following parameters are recorded.

i) Name of the product

ii) Code mark

iii) Can size (A1 tall, A2 ½, A10 etc.)

iv) Gross weight

v) Physical condition of the can like mechanical defects, pin holes, swell etc

vi) External seam dimensions

Incubation: The cans are incubated at different temperatures depending on the pH category as below before further examination. Incubation facilitates the multiplication of the surviving organisms, which helps in the subsequent examinations.

Fruit products (pH 4.5 and below): 37° C for 3 days

Vegetable products: Some at 37° C and the remaining at 55° C for 3 days

The cans are examined periodically during incubation and if any cans are swollen, they are removed. After incubation, the cans are cooled before opening.

Opening the Can and Sampling

The cans have to be opened under sterile/ aseptic conditions using sterilizes implements and gas flame. Special type of punches and openers are available for the purpose. Sample is drawn out using sterile glass tubing into a sterile container.

Sample Examination

The sample is used for direct microscopic examination, culturing, pH determination etc. The can under examination is emptied and observed for it's inside appearance and the seam dimensions are examined.

pH determination is important when flat- sour is suspected. As a routine procedure, the net and drained weight of the can is determined and the product quality is visually examined. Test sample should never be tasted. Combustible nature of the gas (hydrogen) in swollen cans can be checked by puncturing the can and directing the gas to a flame. The can interior of hydrogen swelled cans will show heavy corrosion. In the case of sulphur stinker, the can interior will show black or purplish black stain.

Direct Microscopic Examination

The sample taken from the can is smeared on glass slides and stained with methylene blue or carbol fuchsin and examined under the microscope for general morphology of the organisms. Separate slides are also Gram-stained.

The presence of Gram-positive rods suggests under processing while cocci, yeasts etc., suggests leaker spoilage. If spoilage is due to bacteria surviving heat process, not more than one or two types would be present except in gross under processing. In the case of products having pH above 4.0, container leakage would usually show mixed flora. The presence of micrococci and/or yeast is a sure evidence of container leakage. Based on the information, preventive measures such as increasing the process time if under processed or rectification of the can seam if leakage is the cause are undertaken.

Culturing of the can contents is required to identify the microorganisms for further investigations on the spoilage. This is an elaborate process and is not carried out in routine quality control work.

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Which are the major parameters included in the microbiological examination of water?

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2. Explain the procedure for determining 'Plate count'.

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3. Describe the procedure for determining 'Coliform count'.

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4. Describe the 'Swab-rinse' method for assessing surface sanitation.

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5. Describe how a spoiled can is examined for spoilage.

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6. How does microscopic examination of the contents of spoiled can help in preventing subsequent spoilage?

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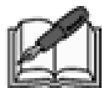


5.6 LET US SUM UP

As you have learnt, food quality assessment is based on physico-chemical, microbiological and sensory methods. Sensory methods for quality assessment are described under a separate unit.

Analytical food chemistry makes use of physico-chemical methods for determining the composition of foods. In food analysis, proximate analysis is routinely carried out to determine the proximate composition of foods. Proximate composition gives a general idea of the major components of foods. In ultimate analysis, individual compounds of the food are determined.

Many of the analytical methods commonly followed in food analysis are described in the Practical Manuals, which you will be performing. A few of those physico-chemical methods, which could not be covered in the practical Manuals, like determination of 'Crude fat', 'Protein', Pectin' and 'Tannins' are described in this unit.

Crude fat and protein are important parts of the proximate composition of foods. You have already learnt the nutritional importance of fats and proteins. Crude fat includes all fat-soluble constituents like tri-glycerides, phospholipids, sterols, essential oils, fat-soluble vitamins and pigments. Therefore, crude fat extracted from foods is used for determining those constituents also.

Microbiological quality of water used in food processing is very important from the food safety point of view. Potable water should conform to certain microbiological quality standards. The major parameters are plate count, coliform count and faecal Streptococci count. If water from a particular source is found unsafe, it should be treated suitably.

In the food processing industry, surface sanitation of the machinery, utensils etc. used should be thoroughly sanitized before use. The method followed to assess the efficiency of sanitation viz. Swab Rinse method is described in this unit.

Spoilage of foods cause monetary loss and may result in public health hazards. Canned low acid foods are of particular concern. Canned foods are generally spoiled either due to under processing or can leakage. The general procedure

for examining microbiological spoilage in canned foods include, proper sampling of cans, physical observation of the cans, incubation at specified temperatures, opening the cans under sterile/ aseptic conditions, sampling the contents of the can (spoiled food material), microscopic examination and some times culturing the sample. Microbiological examination of spoiled cans helps in preventing their recurrence.

5.7 KEY WORDS

Proximate analysis : Nutritional and biochemical composition.

Ultimate analysis : Analysis of specific compounds and elements.

Crude fat : Lipids and fat-soluble components.

Tannins : Flavonoids and cinnamic acid derivatives.

Presumptive test : Tentative or unconfirmed test.

Flat sour spoilage : Spoilage in cans with acid formation without Bulging.

Hard swell : Can spoilage with hydrogen swell and heavy corrosion.

Under processing : Insufficient heat processing of cans.

5.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:
   - Nutritional and biochemical composition
   - Detailed analysis, analysis of individual compounds
2. Your answer should include the following points:
   - Tri-glycerides, phospholipids, essential oils, sterols, fat-soluble vitamins
   - Soxhlet apparatus
   - Ether extraction
3. Your answer should include the following points:
   - Acid digestion
   - Micro-Kjeldahl distillation method
   - % Protein = % Nitrogen × 6.25
4. Your answer should include the following points:
   - Acid extraction of pectin
   - Precipitation as calcium pectate
   - Gravimetric determination
5. Your answer should include the following points:
   - Antioxidant
   - Health benefits

- Oxidation of tannins by potassium permanganate

Check Your Progress Exercise 2

1. Your answer should include the following points:
   - Total count
   - Coliform count
   - Faecal Enterococci count.
2. Your answer should include the following points:
   - Nutrient agar
   - Ringers solution
   - Plating
   - Incubation
   - Counting
3. Your answer should include the following points:
   - Presumptive test
   - Confirmed test
   - Completed test
4. Your answer should include the following points:
   - Cotton swab
   - Ringers solution for rinsing
   - Plating
   - Incubation
   - Counting
5. Your answer should include the following points:
   - Can sampling
   - Incubation
   - Can opening
   - Sterile conditions
   - Product sampling
   - Microscopic examination
6. Your answer should include the following points:
   - Spore formers
   - Gram-positive
   - Micrococci
   - Yeasts
   - Under processing
   - Leakage

5.9 SOME USEFUL BOOKS

1. Manual of Methods for the Examination of Water, Sewage and Industrial Wastes (1963) Special Report No. 47, Indian Council of medical Research, New Delhi.

2. National Cannerd Association (1968) Laboratory Manual for Food Canners and Processors, Vol. 1, The AVI Publishing Co., Conneticut, USA.

3. Official Methods of Analysis (1980) Association of Official Analytical Chemists, Washington, DC 20044.

4. Ranganna, S. (2000) Handbook of Analysis and Quality Control for Fruit and Vegetable Products, Tata McGraw-Hill Publishing Co., Ltd., New Delhi.

5. Recommended Methods for the Microbiological Examination of Foods (1958) American Public Health Association Inc., New York.

UNIT 6 SENSORY ANALYSIS OF FOODS/ BEVERAGES

Structure

6.0 Objectives
6.1 Introduction
  Definition
6.2 Application
6.3 Conducting Sensory Tests
  Identification of Problem
  Selection of Method
  Panellists
6.4 Factors Causing Bias in Sensory Test
  Errors due to Physiological Factors
  Psychological Factors
6.5 Physical Set Up for Conducting Sensory Test
  Laboratory Set Up
  Consumer Testing
6.6 Sensory Test Methods
  Analytical Tests
  Affective Tests
6.7 Sensory Test and Instrumental Measures
  Visual Examination
  Texture Examination
  Olfactory – Sense of Smell
  Gustatory – Sense of Taste
  Flavour
6.8 Let Us Sum Up
6.9 Key Words
6.10 Answers to Check Your Progress Exercises
6.11 Some Useful Books

6.0 OBJECTIVES

After reading this unit, you should be able to:

- differentiate between sensory analysis carried out by a trained panel and casual testing by consumers; and
- explain the methods that should be used in the two situations as both are important in analysing food/ beverage.

6.1 INTRODUCTION

Food / beverage quality is composed of four major components. They are: (i) Intrinsic factors as nutrition, safety which are hidden, (ii) economic factors as investment and profit for a producer (iii) sensory quality noticed by the people as colour, appearance, taste, flavour etc. and (iv) affective quality which decides its ultimate acceptance and use due to like/dislike to a product. The producer is most interested in the last two components to see repeat purchase and good sale of his product. Definite methods to test the intrinsic quality and cost benefit analysis are available for the first two components. These involve human responses and are often confused as one and same. At the outset it should be understood that the two are different. The sensory component testing refers to stimulation of sense organs (Iyendria) and it purely refers to responses

in analytical way. The consumer or affective test refers to response by a population, their like/dislike. It is a test for acceptance or rejection, preference or liking of a product (Indria) and not critical analysis.

The senses used in analysis of foods/ beverages by people are, the eye, which includes colour and appearance, the nose for the sense of smell/ aroma as felt by smelling or while swallowing the food, and by the tongue and interior of the mouth for the four basic tastes of sweet, sour, salt and bitter. The teeth while biting and feel on the tongue or by finger is another important sensory quality of food, as for example, softness of fruits, crispness of biscuit and papads etc. Figure 6.1 gives the idea of the sensations that foods create.

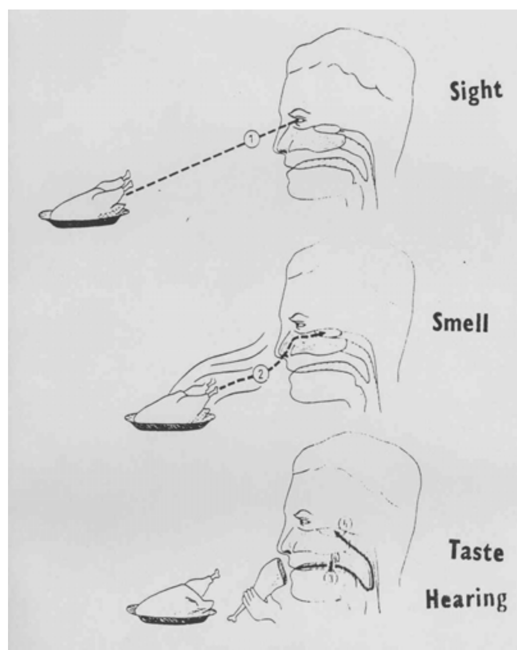


Figure 6.1: Sensory impressions by food

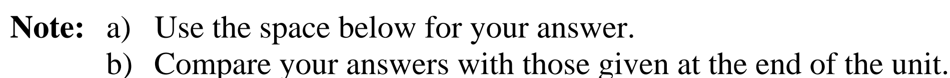
6.1.1 DEFINITION

Sensory analysis is a scientific discipline used to evoke, measure, analyse and interpret the reactions to food as perceived by sense of sight, taste, smell, touch and hearing. The sensory analyst will be equal to an analytical instrument, and will use his 'Iyendrias' (senses) as a tool. It is the 'Iyendria Moulya Maapan' only and not referring to personal likes and dislikes.

The ultimate use of food/beverage is achieved when people consume it. All human beings are endowed with sense organs and use them to judge colour, appearance taste, flavour and texture of food automatically. Is it not true we all look at the food we eat at home or outside for its sensory quality? Think and remember!!

However, this being a psychological response, it can be influenced by several factors. It is therefore important to test the product under careful conditions and in a scientific way.

Check Your Progress Exercise 1



Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. What are food qualities tested by people as tools called?

   a) Intrinsic quality test
   b) Economic quality test
   c) Sensory quality test
   d) Affective (consumer) qualities test

2. Give the definition of Sensory Analysis of foods.

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6.2 APPLICATION

Sensory analysis tests are used in foods/beverages, personal care products as cosmetics, perfumes, and also in textiles for examination of texture, smoothness, etc. Sensory tests are needed in production, research centres and in consumer studies as in market research or survey. We all have seen sensory quality discussed in basmati rice, instant coffee, shampoo, soap etc.

6.3 CONDUCTING SENSORY TESTS

We know sensory tests are carried out by a panel leader and can be either analytical test done by trained people or as affective test done by consumers. The following remain common to both.

6.3.1 Identification of Problem

The first factor to be considered by panel leader is to identify the problem to be looked into i.e., aim of the test should be known. Since sensory organs are simulated simultaneously, for example, aroma and taste, colour and appearance, etc. the objective of testing has to be clear and focussed. The judge has to look for these attributes only.

6.3.2 Selection of Method

Sensory analysis testing for food quality valuation has been in practice from a long time. It has been used for complex products as tea and coffee, whisky by special blenders and also for simple products as sweetness by flow calorie sweeteners. The choice of test method, however, is very specific to the objective of testing. International and national standards are now available to use uniform pattern of testing. This is given in detail under 4.6.

6.3.3 Panellists

Panel Selection

The panel of judges for laboratory sensory testing are usually selected from people with normal sensitivity and not super tasters. They are from office, co-

workers in factory, research group and management staff. They are collected through circulars or personal contacts. People from both sexes are used. The eagerness to participate is the first requirement, as they have to be interested in it. Initial information on age, sex, health status, likes and dislikes, educational background, availability is necessary. In consumer tests, the target population should be selected. We have to see who uses the product and decides to buy it and they have to test it. For example chewing gum flavour is best tested by teenagers and quality of cooking oil by housewife.

Panel Training

In laboratory sensory analysis, the level of training required depends on the type of test used. It is first done to select a group of people who are sensitive to the required level. For example, ability to test off odour has to be done by carefully selected and trained people. Where more intricate analysis is required, more training with examples are used. The panel leader will select the degree of training needed. In consumer tests, no specific training is given to panellists and only method to fill up the answer sheets/ pro forma will have to be explained.

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Sensory tests are done only for foods. Yes No
2. Aim of testing should be clear first. Yes No
3. Panel members should be super tasters. Yes No
4. Which of the tests need more training and why?

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6.4 FACTORS CAUSING BIAS IN SENSORY TESTS

In sensory tests since human subjects are used as tools, care must be taken to use bias-free conditions. Bias means bad influence. Several difficulties can come up which will disturb the analysis and affect the results. They can be due to physiological factors or psychological factors.

6.4.1 Errors Due to Physiological Factors

Errors due to physiological reasons could be the following:

i) *Health:* People with common cold cannot perceive smell and taste. Textural properties, particularly biting quality cannot be judged by people with artificial molar teeth.

ii) Age is another factor which will influence sensory perception. With proper training and motivation it is possible to use panellists of all ages but care must be taken to avoid too young and too old age group.

iii) Adaptation can occur in sensory in nose or tongue when a product is to be tested continuously. For example testing of pungent compounds or bitterness can make a panellist more tolerant of sensation in a product.

iv) Enhancement of sensation can occur in some situations, for example perception of sweetness can be increased with some other substances as sourness. This is commonly observed in testing gooseberries or bitterness in coffee with added chicory.

v) Suppression of taste sensation is the opposite effect of enhancement and commonly observed in foods. This effect will modify the taste sensation. This is observed in combination of pungent and salt tastes or even addition of oil or fat to a food.

6.4.2 Psychological Factors

In sensory testing, psychological factors play an important role. We all know each of us is capable of thinking and imagining. Some common psychological factors, which bias judgement, are as follows.

i) Anticipatory or expectation errors. This occurs due to the panellist starting to test with a pre-conceived idea. Information received earlier can influence judgement. For example, this may happen in testing sweet tasting low calorie foods.

ii) Stimulus error can occur due to other sensory impression in the same product, for example intense colour in fruit juice is considered strong in flavour. Even irrelevant issues can cause bias in judgement as packaging of product can give a bad influence.

iii) Logical error can come up due to the imagination of panellist. For example, cakes with lot of holes may be expected to be soft and spongy or chapathi with brown spots will be considered soft and well cooked.

iv) Halo effect is another situation of bias where the testing is to be done on 3 to 4 attributes and confuse the panellist if he/she is not careful.

In addition to these factors, testing sequence can also cause bias, as for example first sample tested will be seen critically. Over eagerness to perform well in tests or cross talk can influence the test. The panel leader should minimize these factors, which can bias judgement. The panellists should remain calm and interested to concentrate on the test.

In consumer tests, testing place as in a school or shopping area or home may be a bad influence if wrongly selected.

6.5 PHYSICAL SET UP FOR CONDUCTING SENSORY TEST

6.5.1 Laboratory Set Up

Three main areas are required for sensory testing of foods. They should be away from busy corridors and separated by wall or wooden partition. They are: (i) briefing room, where the panellist is told all about the product to be tested, testing method and number of samples (ii) testing room with table to keep the

samples and write the score card. Lighting and ventilation should be good. Each panellist should be separately seated and not in the visual reach of each other (iii) the third area should have sufficient place to keep all plates and cups used in test. All samples should be presented in uniform containers and coded numbers. Figure 6.2 gives a picture of a testing room in a laboratory set up.

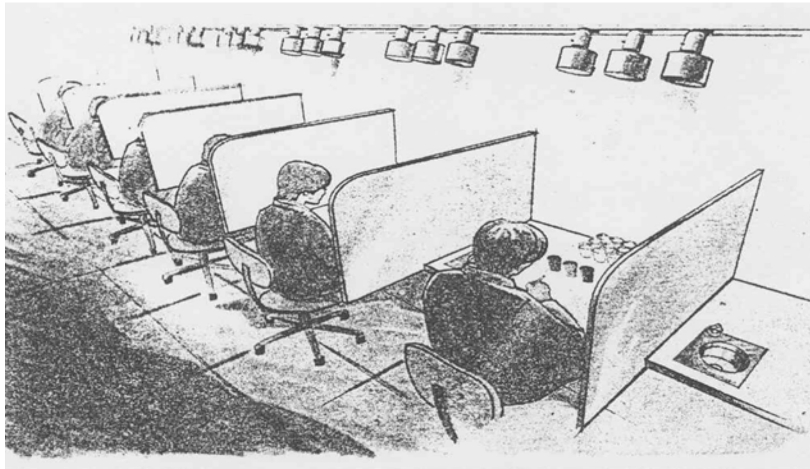

Figure 6.2: Laboratory setup for sensory testing

6.5.2 Consumer Testing

The physical set up depends on product and target group of consumers. It can be a central location testing in shopping area, college campus, hospital, etc.; it can be in club house or homes of friends. Essential requirement in all is to have similar cups and plates with code numbers for all products tested.

Check Your Progress Exercise 3

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Bias in sensory test (wrong influence can arise due to a) Physiological, b) Psychological factors.

Yes No

Give two examples for both.

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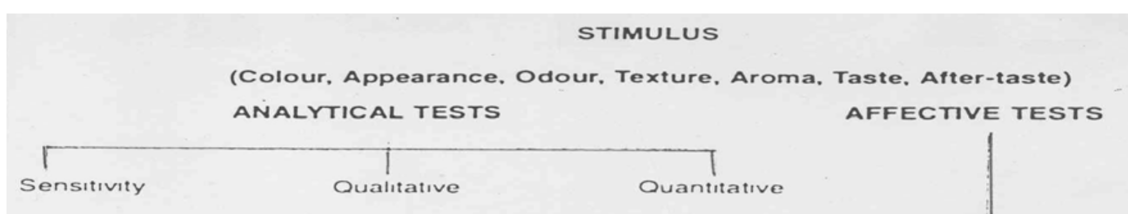
6.6 SENSORY TEST METHODS

Bureau of Indian Standards follows the internationally standardised sensory test methods also. The Table 6.1 gives the test methods adopted. The first distinction is between analytical methods i.e., laboratory test method and second is the affective or consumer test method.

Table 6.1: Sensory testing methods



6.6.1 Analytical Tests

These are the laboratory methods used to test the product for its sensory quality.

Sensitivity Test

This refers to basic difference test. It can be performed as threshold test to identify a sensory impact. Threshold tests are used, for example, to select people with normal sensitivity to sweetness or saltiness. It can be used to detect adulteration Samples are tested in a line till they find out the sensation.

Sensitivity tests can also be done as discriminative test in pairs or triangle. These two-sample tests are simple. We all know it is possible to compare one with just another easily with out confusion. The order of examining, which is first? which is second? should be properly planned to avoid the bias that first is always best. For example think of comparing a new orange drink with fresh juice. Paired test is by testing two samples at same time and see if they are same or different. Figure 6.3 shows how the samples are arranged. You will see 4 pairs of sample arranged for testing. Plain water is used in between samples. Only small quantity of test sample is given under coded numbers.

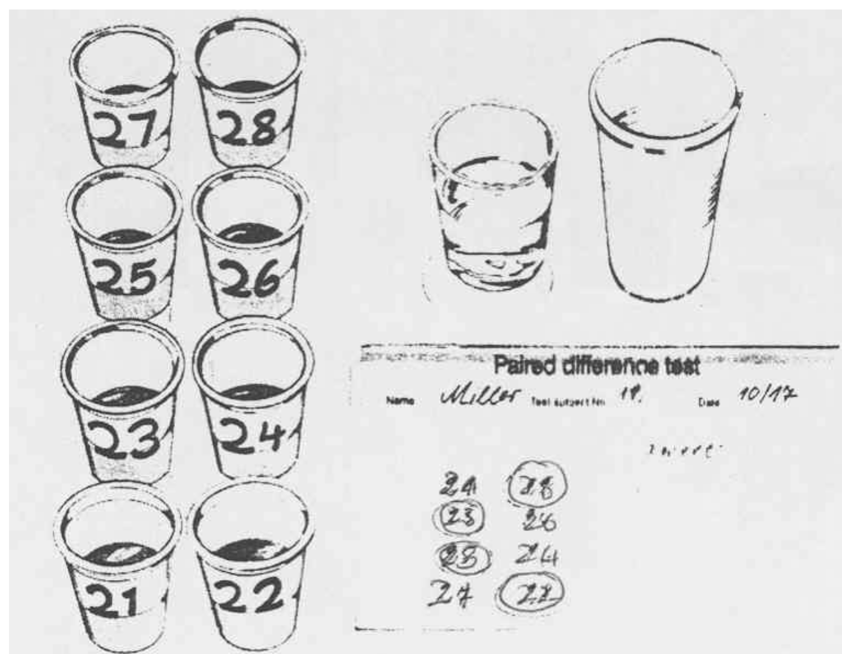


Figure 6.3: Paired comparison test

Minimum 7-10 panellists should examine the samples. Test results are checked for significance of difference by referring to statistical tables.

Triangle test is another method for difference test. The samples are presented in groups of three. Here two are identical and one is odd. The test is to identify the odd sample. For analysing the data, the total number of correct identification is counted and referred to table for significance of difference.

Qualitative Tests

Many times more than two samples will have to be tested for more attributes. For this qualitative tests can be used. This test is useful for working with semi-trained panel of 7-10 members. One of the methods is to arrange the tested foods in the order of increasing intensity (e.g. strong or week aroma), or quality (e.g. good or bad aroma). Ranking is another method. For examples in chocolates, four to five brands can be tested for colour and appearance, texture, flavour and overall quality in one session. The panellist will have to arrange the samples in order of 1st rank for best colour then for best texture etc. Each attribute is tested separately. Classification method is another method. These can be for a specific category of sensory attributes for example as depth of yellowness in pineapple juice. The data in these tests is analysed using non-parametric method. The samples should be tested as coded samples and in random order to prevent bias.

Quantitative Test

In sensory tests to get information on 'how' and 'why' the foods are different, marks for the sensation felt has to be given. The panellists have to be trained more to do these tests and a minimum of 7 panellists are required. They have to be trained both to identify the sensation and give marks to it. This method is very useful for factory and research people. The methods are scoring, rating and time-intensity tests.

In scoring test, numbers as in figures or descriptive scores are used. These scores are useful when the panellists are well trained. For example in a product as jam, ketchup etc. scoring and grading for colour, consistency, set and aroma is useful. Similarly duration of sensory feeling can be matched with time-intensity as in natural and artificial sweeteners. The data is analysed for arithmetic mean and standard error. These quantitative tests can be tested further by detailed statistical tests. Indian standards institution has given details of these tests.

Quantitative description method, popularly called as QDA is another very useful methods for testing foods and beverages. It is a complete and detailed system of panel training, selection of range of products and vocabulary development in simple language. 8-10 well trained panellists can participate. For example description of Dosa or Samosa can be completely obtained and tested by this method. A reference can be maintained as a standard and any number can be tested by a panel. The method can be used to get difference, defects and desirable sensory quality. Recently available computer aided data analysis is very useful for testing.

Check Your Progress Exercise 4

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Which are the two groups in sensitivity tests?

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2. For two samples ……………….. and …………………. tests can be used.

3. Qualitative test can be done with > 2 samples.

Yes No

4. Significance of difference is decided by referring to statistical tables.

Yes No

5. Which tests are used to know 'How' and 'Why' foods are different? What are the methods used to give marks?

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6.6.2 Affective Test

This group of tests is used to get the reaction of the consumer for the food. It tests the users opinion of the product. Three common methods are acceptance, preference and degree of liking. These tests need careful planning and the objective of the test should be known. Question should be asked accordingly. Selection of people who do the tests should be correct, for example baby foods should be tested by mothers and traditional family food should be tested by all at home. Consumer attitude will also change and it is better to check the target population periodically. The number of samples in consumer tests should not be more than two. Consumer response study should never be less than 50 number and 100 will be best. The questionnaire should be as simple and focussed as possible and related to the objective. Consumer tests can be handled for the four types of problems given below:

Acceptance Test

Acceptance is the simplest decision making job for the consumers but only one sample should be tested in one session. They should be asked to concentrate on attribute like for example, overall quality or aroma of new variety of rice, etc Since consumers will relate it to other factors as price, it can be done only at a central location in a shopping complex, college campus, etc., A new product can be tested here. These tests can be done at home also for example, a

new masala mix for rice. Results are given as percentage of people accepting it.

Preference Test

Preference test is another possibility in consumer tests. Not more than three samples should be tested at a time. This can be as 1st, 2nd, and 3rd preference. It should be done by consumers who are familiar with the product. For example cola a type of drink, by college students and instant coffee by office staff.

Hedonic Test

Hedonic test is a direct reference to personal like/dislike to a product. The test is done on 5 or 7 point scale of 'like very much' to 'dislike very much'. The actual users will have to test the food/beverage. New product for example, noodles, shakthi drink etc., can all be tested by this method. Here also a minimum of 100 responses will have to be collected. Figure 6.4 shows one sample of score card for children's food. Here results are as percentage liking it or not.

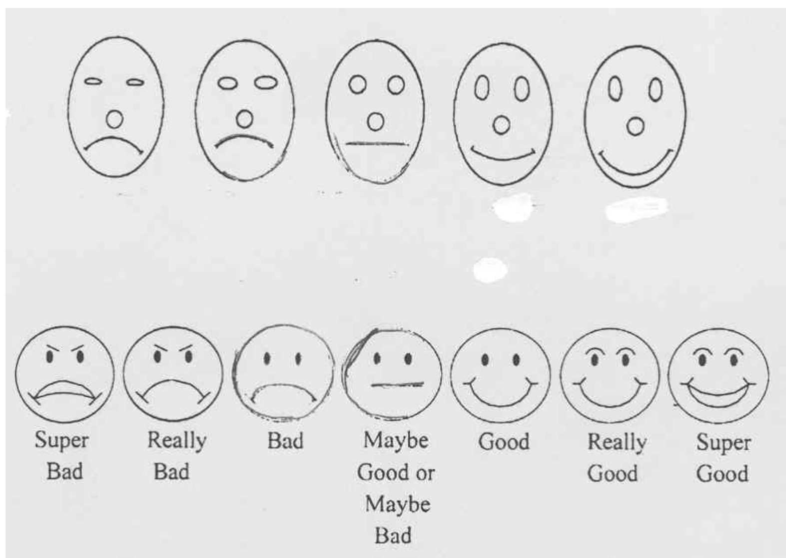

Figure 6.4: consumer evaluation card

Check Your Progress Exercise 5

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. How many people should do the consumer tests?

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2. In consumer tests three types are used, they are a) acceptance, b) preference, c) like. What is the opposite of these three?

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6.7 SENSORY TEST AND INSTRUMENTAL MEASURES

The sensory test when carried out by scientific method will be useful to support instrumental tests. The following are some corresponding tests.

6.7.1 Visual Examination

Visual examination is by the rods and cones in the eye where colour, shape, structure, freshness, etc. are observed. The human eye can distinguish up to 1500 different hues/shades of colour. For example, degree of colour in fruits from green to over ripe fruits, the shades of brownness in chocolates, etc. The instrumental measures for matching what the eye can see are in the visual range of 380–780 nm as L*a*b* tristimulus values in CIE system. The average level of difference as seen by people can be used to describe the colour. Shapes of foods are easier to be measured in terms of geometry of height, length, etc. This can be shown as example in cakes that are baked, sweetmeats as jelabi, etc. Degree of freshness can be noticed in some products by checking for bubbliness, in some beverages as soda and orange.

6.7.2 Texture Examination

Auditory (hearing) sensation in foods is caused by stimulation of ear drum by the sound waves. This is called as texture as crispness of papads or fresh fruits as in apple, etc. It is what the teeth feel when biting the food. It is closely related to structure and movement of food in the mouth. There are advanced texture measuring instruments to study the hardness, softness of food and viscosity of beverages.

6.7.3 Olfactory – Sense of Smell

Olfactory or sense of smell is a chemical stimulation of the receptors in the nose and upper oral cavity. Human odour memory is short but can be trained to recall. Up to 150 odour types can be differentiated. The air carries the chemicals that give the smell. It is difficult to understand how nose can pickup differences in mango variety or bad smell due to toxins. Instrumental measures as gas chromatography or headspace volatiles are used to analyse the individual chemicals. Recently electronic nose is developed to match human response.

6.7.4 Gustatory – Sense of Taste

The sense of taste noticed by tongue and parts of oral cavity are developed early in human beings. Preference for sweet taste is seen even in one week old babies. Instruments for measuring salt content, sweetness and sourness are available. The simple ones are Brix (sugar content), sodium chloride content and pH (food acid content). Special methods are also there.

6.7.5 Flavour

Flavour is the common term for the total effect of odour, taste and touch experienced by people. It is a sensation more closely related to aroma when

the food/beverage is put in the mouth. Highly trained panel only can test this. No single instrument can measure this sense.

Check Your Progress Exercise 6

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Sensory tests for colour, shape and texture can be related to instruments.

   Yes No

2. What causes smell in the nose and how do they reach the nose?

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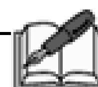
3. No single instrument can measure total flavour test.

   Yes No

6.8 LET US SUM UP



In this unit we learnt that sensory analysis of foods and beverages refers to using humans as instruments/tools. The analytical tests for colour shape, aroma/odour, taste and texture of foods are done by trained people called panellists. Like/dislike, acceptance/rejection are tested by normal consumers is affective tests and done by consumers. Tests have to be carried out under bias free conditions i.e., without wrong influence. Specific methods of testing and analysing data is given by Indian Standards. There are three major types of analytical tests. We need not use any super taster but people like you and me can do the test. There are three types of consumer tests. For analytical tests seven to ten members are needed and for consumer test at least 50 people should be used. For all sensory tests, some instrumental analysis is possible but not for total flavour and acceptance or liking. Only human beings can tell this.

6.9 KEY WORDS

Senses of humans : Eye for Colour and shape, teeth and flow in the mouth for texture and viscosity, nose and back of throat for smell and aroma of food. Tongue for taste.

Analytical sensory Tests : Tests done by trained people.

Consumer tests : Tests done casually in markets, schools etc.

Sensitivity tests : Threshold and two sample tests.

Qualitative test : More than two samples for arranging in order.

Quantitative test : Tests where marks are given.

Iyendria	:	Senses (analytical).
Indria	:	Likes/ dislikes (affective).
Sensory analysis	:	Iyendria Moulya Maapan.
Bias	:	Bad influence.

6.10 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. b) Sensory quality test
   d) Affective quality test

2. Sensory analysis is a scientific discipline used to evoke, measure, analyse and interpret the reactions to food as perceived by sense of sight, taste, smell, touch and hearing. The sensory analyst will be equal to an analytical instrument.

Check Your Progress Exercise 2

1. No
2. Yes
3. No
4. Analytical tests need more training of panellists as the judge has to perform like an instrument.

Check Your Progress Exercise 3

1. Yes

   Examples: Physiological bias- Fever, adaptation
   Psychological bias- Expectation error, cross talk

2. a) Wrong
   b) Wrong
   c) Right
   d) Right

Check Your Progress Exercise 4

1. a) Threshold test
   b) Discrimination test
2. *Paired* and *Triangle* tests
3. Yes
4. Yes
5. Quantitative tests are used to get answers on how and why foods are different. The methods to get marks are, scoring, rating, scaling and time-intensity tests.

Check Your Progress Exercise 5

1. At least 50 people should do the tests.

2. a) Rejection
   b) No preference
   c) Dislike

Check Your Progress Exercise 6

1. Yes

2. Chemicals reaching the nose cause the sensation of smell. They are carried by the air we breathe in.

3. Yes

6.11 SOME USEFUL BOOKS

1. Indian Standards (1968) IS 6273 parts I-III.

2. Shanthi Narasimhan and D. Rajalakshmi (1999), Sensory evaluation of fermented foods, in Biotechnology: Food Fermentation, Vol. 1, Ed. V.K. Joshi and Ashok Pandey: Educational Publishers and distributors, Asia Teca Publishers Inc. New Delhi, p.346-382.

UNIT 7 ANALYTICAL INSTRUMENTATION – ANALYTICAL BALANCE, pH METER & CHROMATOGRAPHY

Structure

7.0 Objectives
7.1 Introduction
7.2 Measurement of Mass
Analytical Balances
Mechanical Single Pan Balance
Electronic Analytical Balance
7.3 pH Measurement – pH Meter
7.4 Chromatography
Classification of Chromatographic Methods
General Principles of Chromatography
Paper Chromatography
Thin Layer Chromatography
Column Chromatography
High Performance Liquid Chromatography
Gas Chromatography
7.5 Let Us Sum Up
7.6 Key Words
7.7 Answers to Check Your Progress Exercises
7.8 Some Useful Books

7.0 OBJECTIVES

After reading this unit, you should be able to:

- explain the principle of measurement of mass;
- describe the different types of analytical balances;
- explain the principle of pH measurement;
- explain the principles of chromatographic techniques; and
- describe the instrument details if gas chromatograph.

7.1 INTRODUCTION

In Unit 3.2.1, you have learnt some of the analytical and quality control methods based on the physical properties of food components. You have also learnt that instrumental methods are becoming the preferred methods over the classical volumetric and gravimetric methods. Some of the instruments are becoming more and more sophisticated and capable of automatic analysis. Even the simple analytical balance used for all analytical determinations has gone through a lot of developments and has become highly complex and refined. Therefore, all those engaged in analysis and quality control of food and food products should have some basic knowledge of the instruments, which they will be using.

In the following two units, you will be learning about a few important instruments. Microscope, a very important instrument, will be dealt with in another unit along with microbiological techniques. Some methods like texture

measurement and reflectance colour measurement require more theoretical explanation to understand. Therefore, they are covered under physical methods in Unit 3.2.1. It is presumed that gadgets and equipments like gauges, hydrometers, ovens etc. are so simple that they can be straight away handled.

In this unit, you will be learning about modern analytical balances, pH meter and chromatography.

7.2 MEASUREMENT OF MASS

In the course of most chemical/ food analysis, an accurate and reliable measurement of the mass (weight) of a sample is required. The terms **'mass'** and **'weight'** are often used synonymously but they are different. Mass is a fundamental quantity which is an invariant measure of the amount of matter in an object. Weight is the force of attraction that exists between an object and its surroundings, mainly the earth. Therefore, the weight of an object varies with the altitude of the place where it is weighed. For example the weight of a coin is less in Shimla than in Mumbai whereas the mass of the coin remains constant in both the places. Weight and mass are related by the expression:

$$W = M g$$

Where, **W** is the weight of the object, **M** is its mass and **g** is the acceleration due to gravity. In order to free the results from dependence upon altitude, a **balance** compares the weight of an object with the weight of a set of standard mass. Because g affects both unknown and known masses equally, an equality between their weights indicate an equality in mass. Therefore, measurement of mass is usually referred to as weighing and the results of weighing are called weight.

7.2.1 Analytical Balances

An analytical balance is a weighing instrument, which has a maximum capacity ranging from 1g. to a few kilograms but usually 160 to 200 g. Weighing can be made with a precision of ± 0.1 mg. Semi micro analytical balances have a maximum loading of 10 to 30 g. with a precision of ± 0.01 mg. and micro analytical balances have a capacity of 1 to 3g and precision of 0.001 mg. The analytical balance has undergone significant evolution during the past several decades. The traditional analytical balance had two pans suspended from the two ends of a lightweight beam, which was supported on a knife-edge. Single pan balance is more convenient to use and hence has almost completely replaced the double pan balance.

7.2.2 Mechanical Single Pan Balance

The two types of balances, however, have several common fundamental features. The components of a mechanical single-pan balance is shown below (Figure 7.1):

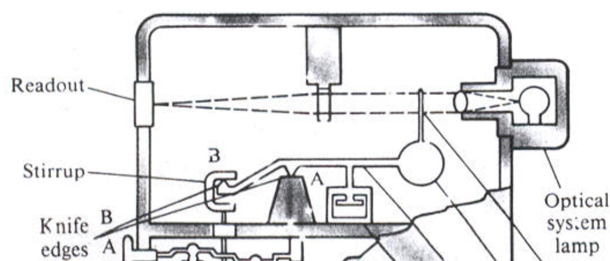


Figure 7.1: Single-pan analytical balance

A lightweight beam is supported on a hard surface (agate or synthetic sapphire) by a knife-edge A. At the left end of the beam there is one more knife-edge B from which a pan for holding the object to be weighed and a full set of weights held in place by hangers is attached. These weights can be lifted from the beam, one at a time, by a mechanical arrangement that is controlled by a set of knobs on the exterior of the balance case. At the right end of the beam a counter weight is fixed which just balances the pan and the weights on the left end of the beam.

The performance of a mechanical balance is critically dependent upon the perfection of the knife-edges. They should permit motion of the beam and pan with minimum friction. To protect the knife-edges from damage and ware when an object is placed on the pan or when the balance is not being used, the balance is equipped with a beam arrest and a pan arrest.

An air damper is mounted near the end of the beam opposite the pan. This device consists of a piston that moves within a concentric cylinder. As the beam is set in motion, air in the cylinder undergoes expansion and contraction causing opposition to the motion bringing the beam rapidly to rest. When there is no object in the pan, the beam will be in horizontal position. When an object is placed in the pan, the beam is displaced downward. Weights are then removed one by one from the beam until the imbalance is less than 100 mg. The angle of deflection of the beam with respect to the horizontal position is directly proportional to the mg. of additional weight that must be removed to restore the beam to its original position. The optical system measures this deflection and converts the angle to milligrams. A small transparent screen mounted on the beam has 1-100 mg. markings. A beam of light passes through the scale to an enlarging lens which in turn focuses a small part of the enlarged scale on to a frontal glass plate located on the front of the balance. A vernier makes it possible to read this scale nearest to 0.1 mg. Before the weighing starts, the vernier is adjusted so that the scale reading is zero.

7.2.3 Electronic Analytical Balance

The electronic balance has neither a beam nor knife-edges. The diagram of an electronic analytical balance is shown below (Figure 7.2):

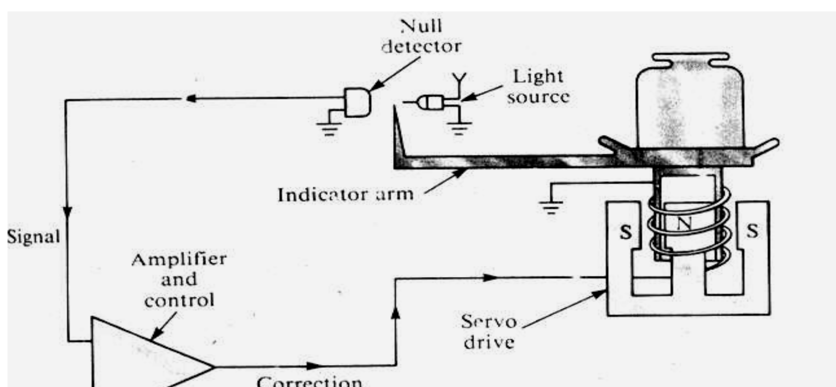


Figure 7.2: Electronic analytical balance

The pan of the balance on which the object to be weighed is placed rides above a hollow metal cylinder which is surrounded by a coil and fits over the inner hole of a cylindrical permanent magnet. An electric current in the coil creates a magnetic field, which levitates the cylinder, the pan and indicator arm. The current is so adjusted that the level of the indicator arm is in null position when the pan is empty. When an object is placed in the pan, the pan along with the indicator arm move downward, which increases the amount of light striking the photocell of the null detector. The increased current from the photocell is amplified and fed into the coil, creating a larger magnetic field, which returns the pan to its original null position. A device like this, in which a small electric current causes a mechanical system to a null position, is called a servo system. The current required to keep the pan and the object in the null position is directly proportionate to the weight of the object and is readily measured, digitised and displayed. Electronic balance is calibrated with a standard mass.

7.3 pH MEASUREMENT – pH METER

You have learnt about the importance of pH in food analysis, food preservation as well as its role in the sensory quality (flavour and taste of foods). You also know that precise maintenances of pH of biological systems is essential for their survival. Therefore, an accurate measurement of pH is of great importance. Before you learn the instruments for measurement of pH it is important to have a clear understanding of pH itself.

Pure water undergoes self-ionisation as follows:

$$2H_2O = [H_3O^+] + [H^-]$$

H_3O^+ is essentially $H^+ + H_2O$

Since the extent of ionisation is negligible and hence the concentrations of $[H_3O^+]$ and $[OH^-]$ are also negligible, the ionisation constant

$$Kw = [H_3O] + [OH^-]$$

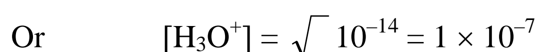
Taking the negative logarithms of both sides

$$PKw = pH + pOH$$

Kw has a value of 1.0×10^{-14} at 24°C (it increases slightly with increase in temperature, e.g. At 100°C the value is 49×10^{-14})

Since $[H_3O^+] = [OH^-]$

$$Kw = [H_3O^+]^2$$



Or $[H_3O^+] = \sqrt{\ } 10^{-14} = 1 \times 10^{-7}$

Therefore, $pH = -\log(1.00 \times 10^{-7}) = 7.00$

At 24°C, any solution whose hydronium ion concentration exceeds 1.00×10^{-7}M i.e. 1.0×10^{-6}, 1×10^{-5} is acidic and any solution whose hydronium ion concentration is less than 1.00×10^{-7} M i.e. 1×10^{-8}, 1×10^{-9} is basic.

pH Meter

pH meters are based on the principle of potentiometry. Potentiometry is the measurement of potentials or voltages of electro chemical cells. An electrochemical cell has two electrodes namely a reference electrode and an indicator electrode or working electrode. An electrode potential of a reference electrode is known and is independent on the composition of the solution under study. The potential of the working electrode employed in conjunction with the reference electrode on the other hand is dependant on the concentration of the solute under investigation. The third component of an equipment required for potentiometric measurement (including pH measurement) is a potential measuring device.

There are three important reference electrodes. They are (1) Standard Hydrogen Electrode (SHE), (2) Saturated Calomel Electrode (SCE) and (3) Silver − Silver chloride Electrode. Even though SHE is the ultimate reference against which the potentials of all other electrodes are measured, it is not convenient to use for routine purposes. Therefore, the later two are often used. The indicator or working electrode for pH measurement is the glass membrane electrode.

7.3.1 Glass Electrode

Glass electrode is a type of ion-selective electrode (ISE). ISEs permit selective determination of numerous cations and anions. The basic principle of glass electrode for the measurement of hydrogen ion concentration is the measurement of the potential that develops across a special type of thin glass membrane that separates two solutions having different hydrogen ion concentrations. Figure 7.3 shows a typical glass electrode.

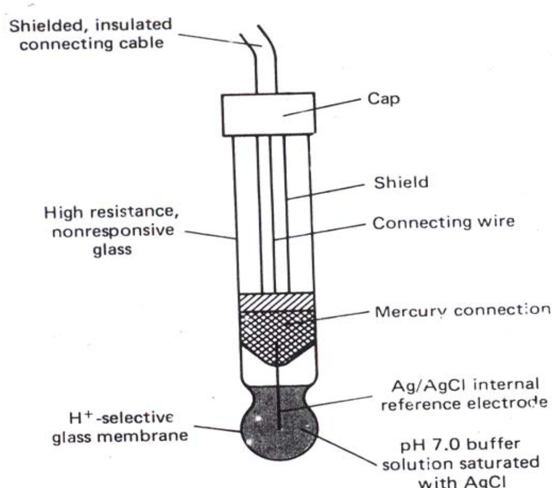


Figure 7.3: Glass electrode

The glass electrode consists of a thin, pH sensitive glass membrane sealed onto one end of heavy-walled non-responsive glass or plastic tube. The internal element consists of silver-silver chloride electrode immersed in a pH 7 buffer saturated with silver chloride. Some glass electrodes contain dilute hydrochloric acid saturated with silver chloride. A silver wire in this solution forms a silver/silver chloride internal reference electrode. The complete system consists of a glass electrode with an internal reference electrode, calomel or silver/silver chloride reference electrode and a potential measuring device. Although, the internal reference electrode is part of the glass electrode, it is not the pH sensing element. Instead it is the thin glass membrane at the tip of the electrode that responds to the pH.

Glass electrodes are available in a variety of sizes and shapes. For example, semi microelectrodes are so small that they can be used to measure the pH of solutions as little as 0.2 ml. There are also electrodes in which the glass electrode and a reference electrode like silver/silver chloride electrodes are physically combined and encased in a single tube. These **combination electrodes** have the advantage of being more compact than a separate two-electrode system. Figure 7.4 shows a diagram of a combination glass pH and silver/silver chloride reference electrode.

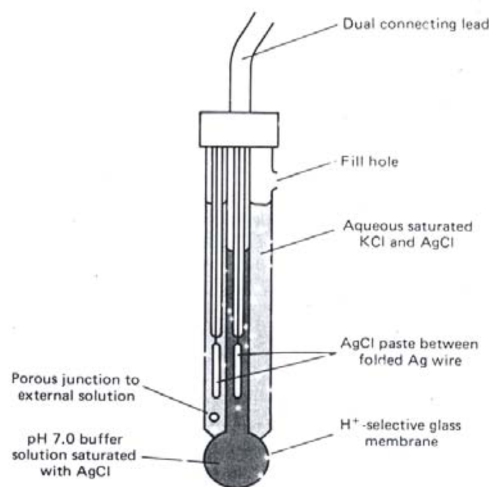


Figure 7.4: Combination pH electrode

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. What is the difference between mass and weight?

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2. Describe the essential components of a mechanical single pan analytical balance.

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3. How does an electronic balance differ from a mechanical balance?

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4. Why the pH of pure water is 7.0?

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5. Describe the main components of a pH meter.

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7.4 CHROMATOGRAPHY

Chromatography is a method of separation of the components of a mixture of closely related compounds and depends upon the distribution of these components between two immiscible solvents. The separated components are detected usually as such or after converting them to coloured complexes. Other signals such as thermal conductivity, visible, ultraviolet and infrared absorption, refractive index and electrical conductance have also been employed to complete the analysis. There are a number of chromatographic separation techniques, which you will be learning in this section.

Tswett is credited with the major part of the discovery and description of chromatography. Since the initial studies were on the separation of coloured components, the method came to be known as chromatography, which means **'description of colours'** or **'writing in colours'.** However you will be learning that present day chromatographic methods are not restricted to separating coloured substances.

Even though the basis of chromatography was laid in the beginning of the 20th century, it was only in 1941extensive work on chromatography was carried out and it's vast potential was realised. This was mainly sue to the work of Martin and Synge who got the Nobel prize in 1952 for their contribution in the field. They did the developmental work on liquid/liquid partition chromatography using inert adsorbents in columns and also paper (inert cellulose) chromatography. Another development in chromatographic technique, which took place during the middle and later 1950s, is the thin layer chromatography (TLC). Stahl is credited for most of the early developmental work on TLC. Martin and Synge even suggested that there is no reason why the moving phase in chromatography should not be a gas. They used a moving gaseous phase to separate a mixture of fatty acids on a conventional liquid chromatography column. Thus gas chromatographic technique was born which has developed into a very powerful analytical tool today. However, it has to be accepted that the modern gas chromatography owes a lot to the development of high sensitivity detection systems.

7.4.1 Classification of Chromatographic Methods

In any chromatographic method, the components to be separated are distributed between two phases. One of the phases is the stationary phase and the other flows through or along the stationary phase and is called the mobile phase. The mobile phase carries along with it the mixture of compounds to be separated. The mobile phase can be either liquid or gas while the stationary phase can be a solid or liquid. **Adsorption chromatography** is in which the stationary phase is a solid and the mobile phase is a liquid. Most of the column chromatographic methods are based on adsorption. The separation process in which both the stationary and the mobile phase are liquid is called **partition chromatography.** Even column chromatography and paper chromatography in which a liquid stationary phase (mostly water) is held on the inert solid support (column filling material or paper) are basically partition chromatography. However it has to be noted that the overall separation process in liquid/ liquid chromatography is most likely due to a combined effect of adsorption and partition.

When the mobile phase is a gas, two possibilities similar to the above are possible. One is in which the stationary phase is a solid and is called **gas – solid chromatography**. The second, which is more popular, is called **gas – liquid chromatography (GLC)** in which the stationary phase is a liquid held on an inert material. Due to the wider application of GLC, quite often it is referred to as just gas chromatography (GC).

Thin layer chromatography (TLC) can also be based on liquid/ liquid or solid/ liquid principle. Somewhat unrelated separation processes viz. ion exchange chromatography and exclusion chromatography are also often included under chromatography. A schematic representation of different chromatographic methods is shown in the Figure 7.5.

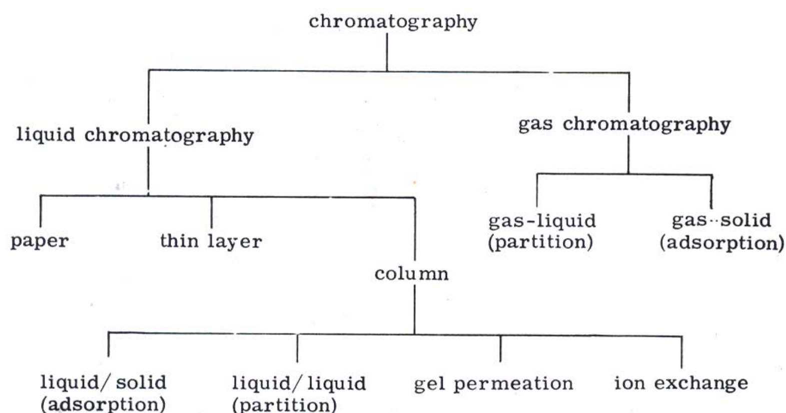

Figure 7.5: Classification of chromatographic methods

7.4.2 General Principles of Chromatography

A sample of a mixture of solutes applied to the stationary phase of a chromatographic system (column, paper or TLC) is carried by the mobile phase (liquid or gas) through the stationary phase. Depending on the nature of the mobile phase, the transfer of components from the stationary phase to the mobile phase is due to one or more of the following factors.

i) adsorption on to the stationary phase,

ii) partition into the liquid stationary phase,

iii) formation of polar bonds with ionic components of the stationary phase.

Separation of the components of a mixture by chromatography is based on the principle that the speed of movement of an individual component through the stationary phase depends on its partition between the two phases. The partition coefficient of each component depends on its sorption characteristics under the experimental conditions. If the sorption characteristics of the individual components of a mixture differ significantly, they move through the chromatographic system at different rates resulting in their separation.

The chromatographic behaviour of a solute is described generally by its retention factor (R). In chromatographic work, the retention factor is termed as Rf value.

$$Rf = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the mobile phase}}$$

Rf value of a substance is more or less constant for a fixed set of chromatographic conditions and hence is used as one of the criterion for its identity.

7.4.3 Paper Chromatography

As mentioned already, paper chromatography is essentially liquid/ liquid partition chromatography. The stationary phase is the water, which is held by

the inert cellulose of the filter paper. The mobile phase is a solvent, usually a mixture of solvents saturated with water. Obviously, such a system is suitable for separating hydrophilic compounds and not hydrophobic compounds. For separating hydrophobic compounds, modified filter papers are required. The paper used for paper chromatography is usually Whatman No.1 or No.2. Thicker filter papers are used for separating larger quantities of a mixture for extraction (elusion) and further studies.

General procedure: After selecting a suitable filter sheet of desired dimension, a pencil line is drawn parallel to one edge of the filter paper. Only pencil should be used for drawing the line or for making any markings because ink may dissolve in the solvent. The distance of the pencil line from the edge of the paper depends on whether one is carrying out descending or ascending chromatography. The difference between the two will be explained subsequently. For descending chromatography the line should be about 10 cm. from the edge of the paper and for ascending chromatography about 2 cm. is sufficient. In descending chromatography the pencil line should be drawn in such a way that while placing the paper in the tank assembly it should be after the antisyphon rod.

Application of sample: Sample preparation for different groups of compounds varies considerably. They are quite elaborate and are described in their respective methods available in textbooks or publications. Therefore, they are not included in this section.

Due to the simplicity of the technique, separation of a large number of substances has been achieved by paper chromatography. However, it should be kept in mind that paper chromatography is mostly used as a qualitative method, though in certain cases quantification is possible.

The samples are prepared in such a way that by applying a few **μl** of the sample is sufficient to detect the individual compounds on the chromatogram. The sample of the mixture is usually applied as a spot on the pencil line on the paper and allowed to dry by itself or by using a stream of hot air. Spots of solutions of known standard compounds likely to be present in the sample mixture are also made on the pencil line, each separated by sufficient space.

Development of the chromatogram: As mentioned earlier, there are two types of paper chromatographic techniques. Descending chromatography is one in which the mobile phase is allowed to flow down the paper by gravity. In ascending chromatography the solvent is allowed to rise on the paper. The requirements for ascending paper chromatography are rather simple. After applying the sample on the filter paper it is folded into a cylindrical form. The cylinder is fastened with thread along the sides to retain the shape. Subsequently it is placed in a petri dish containing an appropriate solvent (solvent mixtures for different groups of compounds have been developed) kept on a glass sheet and covered with a bell jar. Care should be taken to see that the solvent level is below the pencil line. The assembly is made airtight by applying grease. The solvent may take several hours to ascend to sufficient height (about ¾ of the paper). Care should be taken not to allow the solvent to reach the upper end of the paper. Subsequently, the paper is removed, and hung on a suitable support to dry. The separated spots are detected using suitable reagents if not coloured.

The assembly required for descending chromatography is more sophisticated. The tank assembly has a trough or boat on the top to hold the solvent into which the sample spotted edge of the filter paper dips. An anchor rod to hold

the paper and an antisiphon rod to prevent siphoning of the solvent down the paper. A simple tank assembly is shown in the following Figure 7.6.

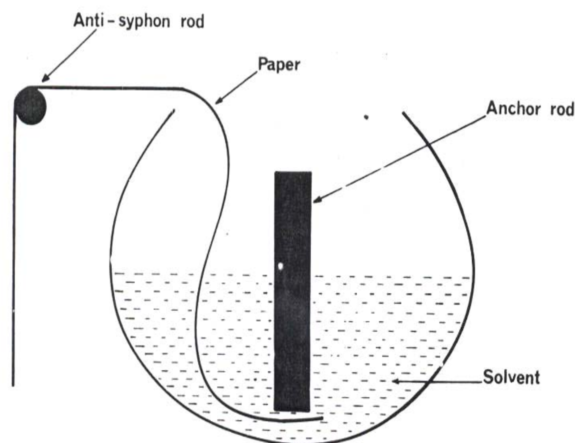

Figure 7.6: Cross section of descending chromatography trough

The rest of the sequence of operations are similar to those explained for ascending chromatography.

Some of the food constituents effectively separated by paper chromatography are: Carbohydrates, amino acids, organic acids, plant pigments, synthetic colours, vitamins, etc.

7.4.4 Thin Layer Chromatography

Thin Layer Chromatography (TLC) is similar to paper chromatography in several ways. The main difference is the nature of the chromatographic sheet. Instead of a filter paper it is a rectangular glass or plastic sheet. On the sheet, a thin layer (usually less than 1 mm.) of an inert material (coating material) like silica gel or alumina is applied. For imparting binding properties, substances like gypsum, polyvinyl alcohol is added to the coating material. For separating different groups of substances, different types of coating materials are used.

For forming a uniform layer of the coating material on the sheet, usually an **applicator** is used. The applicator has adjustment to control the thickness of the layer. The coating material is taken in the applicator as slurry and drawn over a set of sheets to form the layer. A schematic diagram of a TLC applicator is shown in the following Figure 7.7.

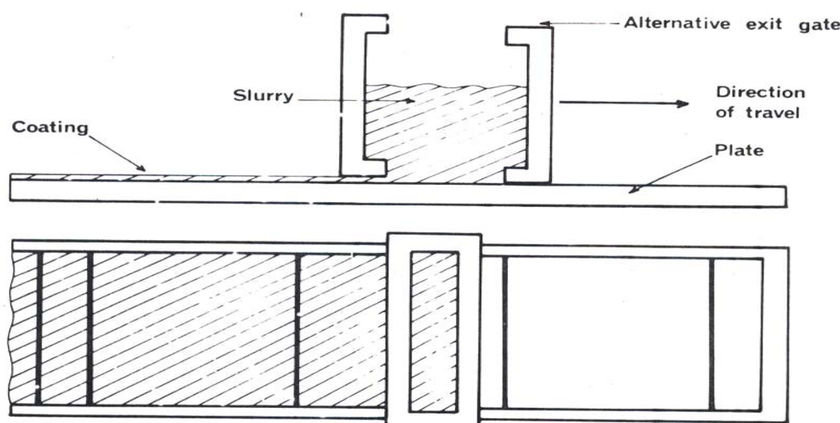

Figure 7.7: TLC applicator

After applying the slurry on the plate, it is allowed to dry in air. For adsorption TLC, the plates are subsequently activated (dried) in an oven at 110°C for 30 minutes or more.

Sample application on the TLC plate is similar to that followed for paper chromatography, except that no pencil line is drawn. The plate after applying the sample and standards spots is developed in a tank using a suitable solvent. The ascending type assembly for TLC is shown in the Figure 7.8.

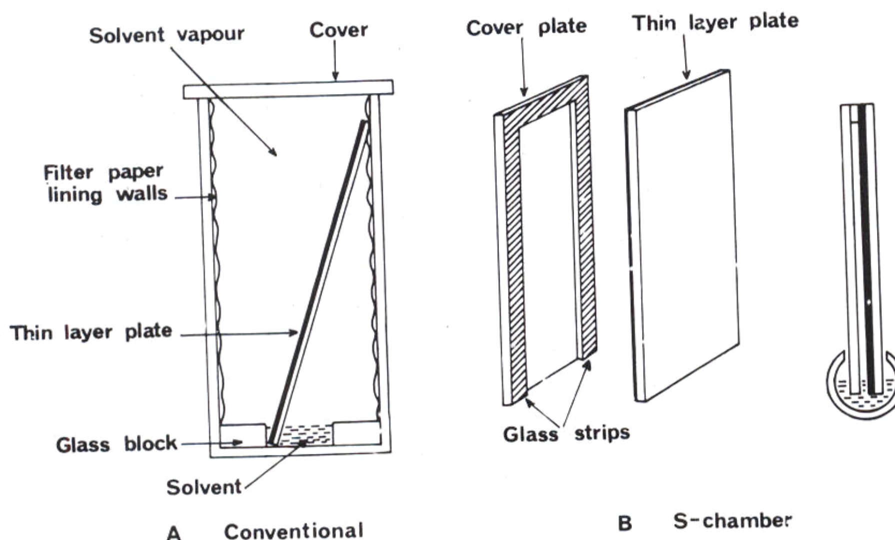

Figure 7.8: Ascending development chambers for TLC

TLC has certain advantages over paper chromatography. They are:

- TLC is far quicker than paper chromatography.
- As the support material used in TLC is truly inert unlike cellulose of filter paper, it is possible to use highly corrosive reagents for detection of components.
- TLC requires much smaller amount of sample and hence more sensitive than paper chromatography.

TLC is widely used for detection and identification of synthetic colours in foods, aflatoxin, pesticide residues besides most other substances mentioned under paper chromatography.

7.4.5 Column Chromatography

Until recently, column chromatography has been mostly employed for sample clean up (purification) prior to analysis by other methods. However, in some cases it is also used as an analytical tool and with the development of high sensitivity detectors, it has now evolved into **high performance liquid chromatography (HPLC)** having several applications.

The basic equipment for column chromatography is very simple. It consists of a vertical glass tube containing the inert material packed fairly firmly. The glass columns used are usually about 30 cm long and 1 cm internal diameter, although much longer and wider columns are used for specific purposes. The lower end of the column is tapered where a sintered disc is fixed or glass wool is placed to prevent the packing material from falling down.

Filling of the column with the packing material is a critical step. Care should be taken to fill the column firmly and evenly. The nature of the column filling

material depends on the particular application. It could be alumina, silica gel, ion exchange resin, Sephadex gels, etc.

The filled column is fixed to a solvent (eluent) reservoir to hold the required solvent. A typical assembly is shown in the following Figure 7.9.

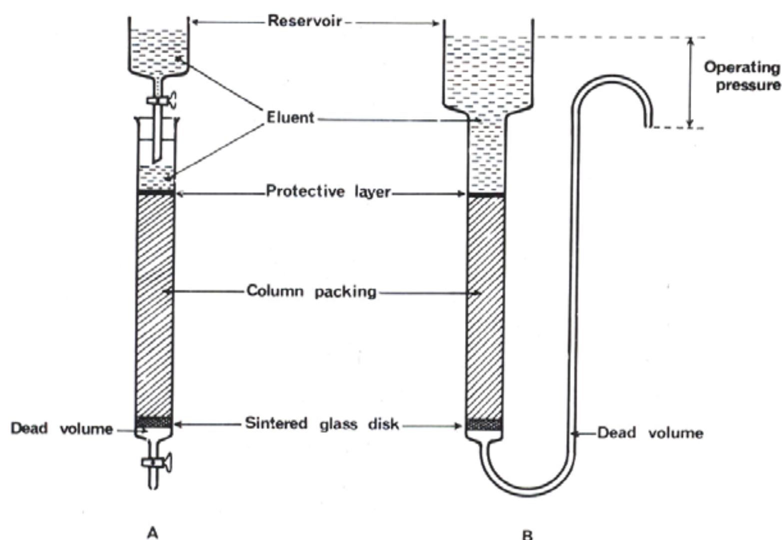

Figure 7.9: Column arrangement for liquid chromatography

After packing the column, it is washed with the intended solvent in order to wash out any impurities that may have been present in the packing material. At the top of the packing, a piece of glass wool is placed as a protective layer.

The sample is applied to the top of the column in a small volume of solvent drop by drop. The sample is allowed to penetrate into the packing material. Flow of the eluent through the column is called **elution.** Elution is normally achieved by gravity, although it may be necessary to facilitate flow by use of pumps or inert gas pressure. Flow rates of eluents for different applications have been standardised. However, flow rates of about 1 ml /min is normal.

For coloured compounds, the movement of coloured bands can be seen and each band can be collected separately in tubes and analysed. For colourless compounds, number of fractions have to be collected and analysed.

A number of methods are available for column chromatographic separation of food components. Separation of carotenoid pigments is an important method followed even today. Amino acids are separated on ion exchange columns. Peptides can be separated on Sephadex columns.

7.4.6 High Performance Liquid Chromatography

In the conventional column chromatography, the flow rate of mobile phase is usually by gravity. Therefore, to maintain satisfactory flow, the particle size of the inert column packing materials cannot be very small. Unfortunately, to get good resolution (separation) of sample mixture, very low particle size packing material is required. Systems have now available which can create pressures of the order of 600 atm. At that pressure, adequate flow rates are obtained even with packing particle as small as 2 to 3 μm in diameter. Along with this development, several detection systems were also adapted for use with liquid chromatographic columns. Liquid chromatography using these high-efficiency columns at high pressures has come to be known as **High-Performance Liquid Chromatography (HPLC)**.

Most HPLC columns are fabricated from stainless steel tubing. Usually the columns are 10 to 50 cm long with an inside diameter of 2-10 mm. The packing material used have diameter in the range of 3 to 10 μm. The detectors used in HPLC belong to one of the four basic principles viz. UV absorption, fluorescence, refractive index or conductance.

7.4.7 Gas Chromatography

In this section you will learn the details of gas-liquid chromatography, which is one of the most widely used analytical technique today. As mentioned earlier, gas-liquid chromatography is usually shortened as gas chromatography or merely GC.

There are a number of reasons why GC has become very popular in analytical work. Firstly, GC permits separation of complex mixtures including those containing closely related homologues and isomers. Secondly, it is relatively simple to prepare samples for analysis because unlike for other analytical methods, GC does not require extensive purification of the sample. The only requirement (may be a draw back) is that the components of the sample should be reasonably volatile.

The instrument used for gas chromatographic work is called a gas chromatograph. The modern gas chromatograph is a very complicated instrument. However, all gas chromatographs have a few key components, which is illustrated in the following Figure 7.10.

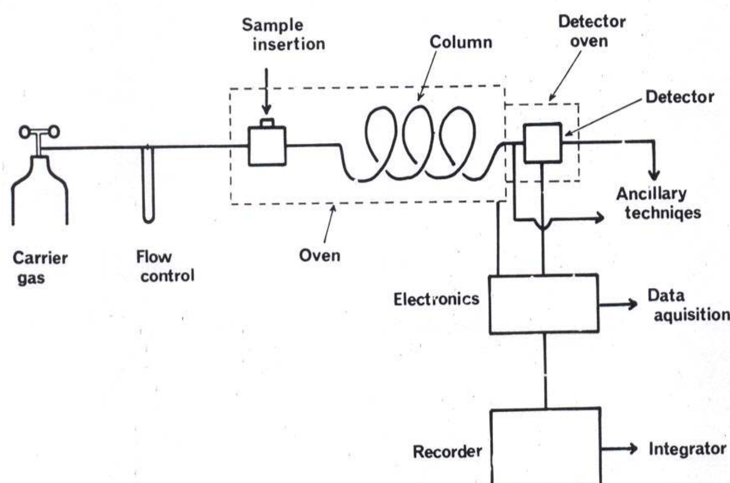

Figure 7.10: Basic components of a gas chromatograph

It can be seen from the diagram that a gas chromatograph has four major components viz. i) carrier gas supply system, ii) sample insertion (injection) system, iii) column assembly, and iv) detector and readout device.

Carrier Gas Supply System

The carrier gas used, which is the mobile phase in GC, must be chemically inert. These include helium, argon, nitrogen, carbon dioxide and hydrogen. The choice of the gas is decided to a great extent by the detector used. The gas supply assembly consists of the gas cylinder and gas flow meters. Gas flow rates play important role in the performance of a GC. Therefore, the gas flow rate is one of the parameters defined in GC procedures. The average gas flow rates for packed columns are in the range of 25 to 150 ml/ min and 1 to 25 ml/ min for capillary columns. You will be learning more about different types of columns subsequently. A soap bubble meter located at the end of the column is used to measure the gas flow rate.

Sample Injection System

Sample injection system has a sample injection port and a vaporizer. For efficient separation of sample components, it is necessary to introduce a small size sample into the column as a ‘plug’ of vapour. Usually a micro syringe is used for the purpose. The volume injected range from <1 to 20 μl. The sample vaporizer is usually maintained at a temperature of 50°C above the boiling point of the least volatile components of the sample. The flow splitter allows the passage of only the quantity of the sample necessary for good resolution and the remaining is let out.

Column Assembly

Two general types of columns are used in GC. They are packed columns and open tubular or capillary columns. Packed columns are made of 1 to 10 m or longer stainless steel tubing having 1.5 to 5 mm internal diameter. Usually they are coiled for compactness. Capillary columns are much longer ranging from 10 to 100 m having very small diameter of the order of 0.1 to 0.5 mm. They are usually made of glass or silica.

The solid support in a packed column serves to hold the liquid stationary phase in place so that as large a surface area as possible is exposed to the mobile phase. The most widely used support material is prepared from diatomaceous earth. The particle size of the support material is usually in the range of 60 to 100 mesh.

Capillary columns are of two basic types viz. wall-coated open tubular (WCOT) and support–coated open tubular (SCOT). In WCOT columns, the inside of the capillary is coated with the stationary phase. In the SCOT columns, the inner surface of the capillary is coated with a thin film of the support material such as diatomaceous earth and over which the stationary phase is applied. Due to coating of the support material, SCOT columns can hold several times more of the stationary phase than in WCOT columns. Therefore, the efficiency of the SCOT columns is better than WCOT column.

Stationary Phase in the Column

The stationary liquid phase used in GC columns (both packed and capillary) are of different types. They are selected based on the nature of the compounds separated on the column. Some of the stationary phase substances are listed below.

Material	Trade name	Common application
Polydimethyl siloxane	OV-1, SE-30	Hydro carbons, steroids, polynuclear aromatics etc.
Poly (phenyl methyl) siloxane	OV-17	Pesticides, glycols, steroids, etc.
Poly (phenyl methyl dimethyl) siloxane	OV-3, SE-52	Fatty acid methyl esters, alkaloids, halogenated comps, etc.
Poly (trifluorophenyl dimethyl) siloxane	OV-210	Chlorinated aromatics, nitro aromatics, alkyl substituted benzenes, etc.
Poly (dicyanoallyl dimethyl) siloxane	OV-275	Poly unsaturated fatty acids, free acids, alcohols, etc.
Polyethylene glycol	Carbowax-20M	Free acids, alcohols, esters, essential oils, glycols, etc.

Detectors

Like in any chromatographic separation process, in GC also, the separated compounds have to be detected and quantified if necessary. An ideal detector should have some of the following characteristics.

- Adequate sensitivity.
- Good reproducibility.
- Wide range of linear response to analyte concentrations.
- Detection with out destroying the sample.
- Response to all types of compounds.

Unfortunately, an ideal detector is yet to be developed. Therefore, for different purposes, different detectors are used. There are a few types of detectors; two of the important ones are described below.

Flame Ionisation Detector (FID)

FID is one of the most widely used detectors for GC. In a flame ionisation detector, the effluent from the GC column is mixed with hydrogen and air and ignited electrically. When organic compounds are burnt at the high temperature, they produce ions and electrons that can conduct electricity through the flame. The current produced is amplified and measured.

Thermal Conductivity Detector (TCD)

TCD is based on the principle that the thermal conductivity of a gas stream is changed by the presence of analyte molecules. The sensing element in a TCD is an electrically heated element (platinum, gold or tungsten) whose temperature at constant electric power depends on the surrounding gas. The thermal conductivities of helium and hydrogen are about six times greater than those of most organic compounds. Therefore, in the presence of even traces of

organic materials, a relatively large decrease in the thermal conductivity of the column effluent takes place. Consequently, the detector undergoes a marked increase in temperature. One major advantage of TCD as against FID is that it is non-destructive meaning that the compounds separated are not disintegrated and hence can be used for further analysis if required. Besides, TCD can detect both organic and inorganic compounds and hence has a wider range.

Signals from the detector are amplified and recorded by suitable electronic instruments. The output in the form of a graph having different peaks for the components is called a **gas chromatogram**. The chromatogram also provides the retention times, height and area of each peak.

Applications of GLC

Even though, GC is an excellent tool for tentative identification of components of a mixture, confirmation of their identity is rather difficult. Therefore, an important trend has been in combining the remarkable fractionation qualities of GC with the superior identification properties of such instruments as **mass spectrometer (MS)**, **infrared (IR)** and **nuclear magnetic resonance (NMR) spectrometers**. Mass spectrometer is very complicated and not meant for routine analytical purposes, hence will not be described in this unit.

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. What is chromatography?

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2. Explain high-performance chromatography.

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3. Enumerate the key components of a gas chromatograph.

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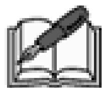

7.5 LET US SUM UP

Modern analytical methods demand very accurate determination of mass. Consequently, considerable developments have taken place in increasing the sensitivity and simplicity of the analytical balance. This resulted in the modern single pan mechanical balance which can weigh with an accuracy even of ± 0.001 mg. Simultaneously, development in electronics have resulted in the evolution of electronic balances which have done away with the knife-edges which are the critical and wearable components of mechanical balances.

Measurement of pH of food systems is very important for various reasons. PH indicators based on colour change of certain chemicals have obvious drawbacks. Potentiometric method for the determination of pH is more reliable. The development of the glass electrode is one of the major breakthroughs in the instrumentation for pH measurement.

Chromatography, which started mainly as a separation technique, has assumed much more importance in food analysis. In addition to the simple techniques like column, paper and thin layer chromatography, gas chromatography and High-performance liquid chromatography have become very powerful tools for qualitative and quantitative analysis of food products.

7.6 KEY WORDS

Mass	:	Invariant measure of the amount of matter.
Weight	:	Force of attraction between an object and earth.
Chromatography	:	Writing in colours or separation of a mixture of compounds based on adsorption and partition.
Partition	:	Distribution of solutes between two phases.
Retention factor	:	Ratio of distance moved by the solute to distance moved by the mobile phase.
Eluent	:	Solvent used as the mobile phase.
Carrier gas	:	Gas used as the mobile phase in gas chromatography.
FID	:	Flame ionisation detector.
TCD	:	Thermal conductivity detector.

7.7 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

- Invariant measure
- Dependent on gravitational force

2. Your answer should include the following points:

- Single pan and set of weights on one end of beam and counter weight at the other end
- Air damper
- Optical system

3. Your answer should include the following points:

- No beam
- No knife-edge
- Servo system

4. Your answer should include the following points:

- Ionisation of water
- Ionisation constant of water
- pH = - log $[H^+]$

5. Your answer should include the following points:

- Glass electrode
- Calomel or silver-silver chloride reference electrode
- Potentiometer

Check Your Progress Exercise 2

1. Your answer should include the following points:

- Separation of the components of a mixture
- Adsorption
- Partition

2. Your answer should include the following points:

- Liquid column chromatography at high pressure
- Electronic detectors

3. Your answer should include the following points:

- Carrier gas
- Sample injection system
- Column assembly
- Detector and readout device

7.8 SOME USEFUL BOOKS

1. Hagris, L.G. (1988) Analytical Chemistry, Prentice Hall, New Jersey.

2. Mac Leod, A.J. (1973) Instrumental Methods of Food Analysis, Elek Science, London.

3. Skoog, D.A., and Leary, J.J. (1992) Principles of Instrumental Analysis, Saunders College Publishing, Florida.

UNIT 8 ANALYTICAL INSTRUMENTATION BASED ON ELECTROMAGNETIC RADIATION

Structure

8.0 Objectives
8.1 Introduction
8.2 Properties of Electromagnetic Radiation
8.3 Spectroscopy
Absorption of Radiation
Atomic Spectroscopy
Refractometry
Polarimetry
8.4 Let Us Sum Up
8.5 Key Words
8.6 Answers to Check Your Progress Exercises
8.7 Some Useful Books

8.0 OBJECTIVES

After reading this unit, you should be able to:

- explain the properties of electromagnetic radiation;
- describe different types of spectroscopic methods;
- describe the components of a colorimeter and spectrophotometer;
- differentiate between atomic absorption and emission spectroscopy; and
- explain the principle and instrumental details of refractometer and polarimeter.

8.1 INTRODUCTION

Majority of the modern instrumental methods of analysis are based on interaction of electromagnetic radiation with matter (elements and molecules). They include emission, absorption, scattering, fluorescence, refraction, reflectance and rotation of radiation. You have learnt reflectance colour measurement of materials like fruits in the previous unit.

Spectrometry is a general term describing various methodologies dealing with the production, use and measurement of electromagnetic radiant energy or radiation. Electromagnetic radiation can interact with matter to produce measurable signals, which are made use of for qualitative and quantitative measurements. In this unit, you will be learning about some of the instruments based on these principles. In order to understand the nature of these interactions, it is necessary to have some basic knowledge of the propagation of radiation.

8.2 PROPERTIES OF ELECTROMAGNETIC RADIATION

Electromagnetic radiation is a type of energy that is transmitted through space at enormous velocities. For simplicity of understanding, the transmission can be viewed to be of a transverse waves consisting of discrete energy packets called photons. The waves oscillate in all the planes perpendicular to the direction of propagation.

There are a few important terminologies associated with the propagation of electromagnetic radiation. They can be explained easily with the aid of the waves of a plane-polarised light shown below (Figure 8.1). Plane polarised light (radiation) is one in which the wave oscillation is only in one plane. You will learn more about polarisation later.

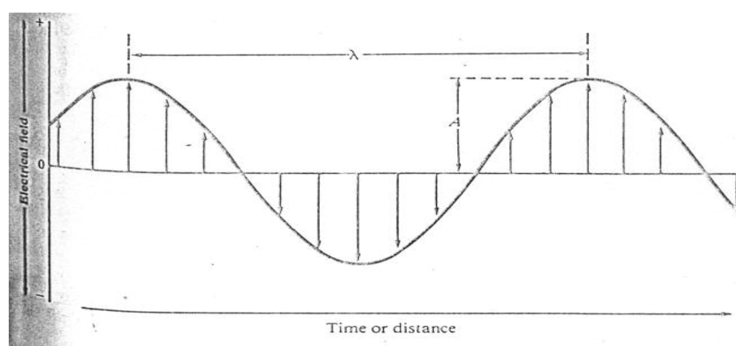

Figure 8.1: A beam of plane polarised monochromatic radiation of wavelength λ and amplitude A

The **amplitude, A** of the wave is defined as the maximum height of the wave. The length of one wave or the distance between two successive maxima or minima of a wave called the **wavelength,** λ of the radiation. The reciprocal of the wavelength, or the number of waves per unit length is called the **wave number, ν.** The time required for one wave to pass a fixed point in space is called the **period, p** and its reciprocal, the **frequency,** υ which is the number of waves passing a fixed point per unit time. A simple relationship exists between the length of a wave (wavelength) and the time required for it to move past a fixed point in space. This relationship says that the product of the wavelength and frequency equals the **velocity of propagation of the wave, c.** In vacuum, the velocity of propagation of radiation (usually referred to as **speed of light**) is approximately 3.0×10^8 m/s. The velocity of radiation in any other medium is always less because of interaction with matter. Since the radiant frequency is invariant and fixed by the source, the wavelength of radiation decreases as it passes from vacuum to a medium containing matter. In air, the velocity is only marginally less (0.03%). Chemists in characterising radiant energy most often use wavelength and frequency.

The power, P of radiation (frequently also called intensity) is the energy of beam that reaches a given area per second. P is related to the square of amplitude, A. Electromagnetic radiation is continuous over all possible wavelengths. It is sub divided according to how the radiation reacts with matter. The visible portion of the spectrum i.e. those wavelengths to which the human eye produces a sensation called colour, is only a tiny part of the spectrum (750 to 380 nm) as shown in the following Figure 8.2.

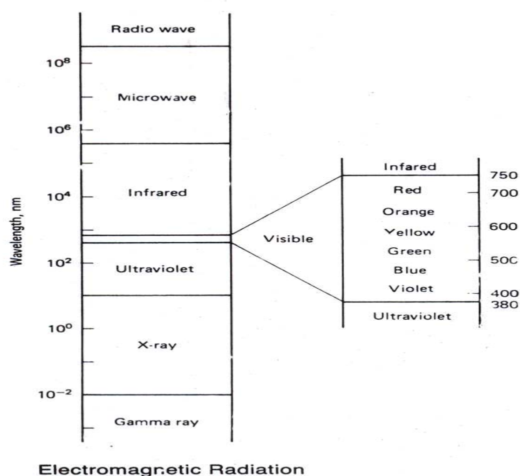

Figure 8.2: The electromagnetic spectrum

You have learnt that the interactions of electromagnetic radiation with matter cause certain responses (phenomena). Optical spectroscopic methods are based on measuring these responses.

8.3 SPECTROSCOPY

Historically spectroscopy referred to a branch of science in which visible or ultraviolet light is resolved into its component wavelengths, thus producing spectra, which were then used for theoretical studies on the structure of matter or for qualitative and quantitative analysis. However, with passage of time, the meaning of spectroscopy has broadened to include other types of electromagnetic radiations also. Currently, spectroscopy even includes studies with other types of radiations including ions (mass spectrometry), electrons (electron spectroscopy), and sound waves (acoustic spectroscopy).

Among various interactions between radiation and matter, absorption of radiation is the most important for analytical work. Visible and ultraviolet radiations are the most widely used for the purpose. Therefore, visible and ultraviolet absorption spectroscopy will only be dealt with in detail here.

8.3.1 Absorption of Radiation

In spectrometry, absorption is a process in which a chemical species in a transparent medium selectively attenuates (decrease the intensity of) certain frequencies of electromagnetic radiation. The absorbing characteristics of a chemical species are described by means of an absorption spectrum, which is usually a plot of some function of the attenuation versus wavelength, frequency or wave number.

In absorption spectrometry, one encounters both atomic absorption and molecular absorption. Atomic absorption relates to absorption of radiation by atoms and ions (especially the metals) and the consequent responses. Methods based on this phenomenon fall under **atomic absorption spectroscopy (AAS)**. The second is called molecular absorption spectroscopy, which has found maximum application in qualitative and quantitative analysis.

Molecular Absorption Spectroscopy

You have learnt that a beam a radiant energy is reduced in power as it passes through a solution containing absorbing substances. The attenuated radiation is transmitted. At this stage it may be of interest to know why a coloured solution has a particular colour. For example a red coloured solution is red not due to the molecules of the solute impart red colour to the solvent. Instead the solute molecules absorb the green component of the white radiation and transmit the red component. Thus, green colour is called the complimentary colour for red. You will learn subsequently in visual colorimetry the complimentary colours are used to measure the absorption characteristics of the coloured solutions.

Wave-length range (nm)	Colour	Complimentary colour
400 – 435	Violet	Yellow – Green
435 – 480	Blue	Yellow
480 – 490	Blue-green	Orange
490 – 400	Green-Blue	Red
500 – 560	Green	Purple
560 – 580	Yellow-green	Violet
580 – 595	Yellow	Blue
595 – 650	Orange	Blue – Green
650 – 700	Red	Green – Blue

Important terms used in Absorption Spectroscopy

The terms can be explained using the simple diagram below (Figure 8.3):

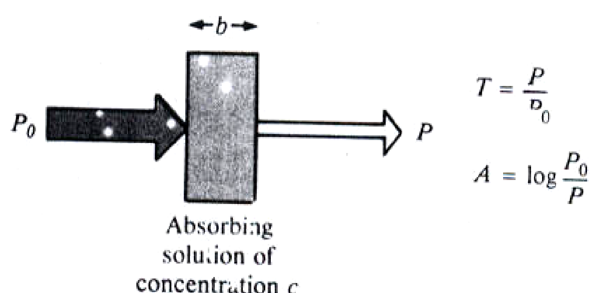

Figure 8.3: Attenuation of radiation by an absorbing solution

The Figure shows a beam of radiation before and after it has passed through a layer of solution with a **thickness, b cm** and a **concentration of c** of an absorbing solution. The power of the beam is attenuated from P_0 to P. The **transmittance, T** of the solution is defined as the fraction of the incident radiation transmitted by the solution.

$$T = P/P_0$$

Transmittance is usually expressed as a percentage (%T).

Absorbance, A of a solution is defined by the expression

$$A = -\log T = \log P_0/P = \log 1/T \text{ or } \log 100/\%T$$

$$A = \log \frac{P_0}{P}$$

$$\Rightarrow \quad A = \log P_0 - \log P$$

Since P_0 always set at 100% and log P is % T

$$A = \log 100 - \log \% P$$

$$A = 2 - \log \% T$$

i.e. $A = 2 - \log \%T$

Please note that in contrast to transmittance, the absorbance of a solution increases as the attenuation of the beam increases.

Relationship between absorption and concentration

The relationship between the amount of radiation absorbed or transmitted and the amount of absorbing substance is called Beer's Law or sometimes Beer's-Lambert Law.

$$A = \log P_0/P = abc$$

Where **a** is a proportionality constant called the absorptivity and **b** is the path length of the radiation through the absorbing media and c its concentration. When the concentration is expressed in moles per litre and b is in cm., they absorptivity is called **molar absorptivity** ε i.e.

$$A = \varepsilon bc$$

Where ε has the units of $l\ cm^{-1}\ mol^{-1}$

Check Your Progress Exercise 1

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Explain the terms wavelength and frequency of electromagnetic radiation.

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2. What is spectroscopy?

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3. Differentiate between molecular absorption and atomic absorption spectroscopy.

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4. Explain Beer's law.

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5. Analyst found that the minimum concentration of 3-aminotriazole (M.W = 84) that could be determined in meat product was 0.05 ppm. At this concentration the absorbance of the coloured reaction product was 0.02 with 1 cm all thickness. What is the molar absorptivity E of this reaction.

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Instruments for Absorption Spectroscopy

Absorption spectroscopic instruments have 5 basic components i) a source of radiant energy, ii) a wave-length selector that allows the radiation of a restricted wave-length region, iii) one or more sample holders, iv) a radiation detector or transducer which converts radiant energy to electrical signal, and v) signal processor and read-out (Figure 8.4).

Figure 8.4: Basic components of a spectrometer

Radiation sources: The basic requirements of a radiation source for spectroscopic studies are (1) should generate a beam of radiation with sufficient power for easy detection and measurement and (2) its out-put power should be stable for reasonable periods. No single source is suitable for all the spectral regions of interest to analytical work. For the visible spectroscopy, the most common energy source is a tungsten filament lamp which has an out-put (325 nm to 3000 nm) which covers the entire visible region of the spectrum and also part of the ultra violet and infrared regions. For ultra violet spectroscopy, the preferred energy source is deuterium discharge lamp which has an out-put in the range of 160 nm to 380 nm.

Wavelength selector: Since the radiation sources are continuous, it is necessary to select the required wavelength at which the measurement is to be made. As it is practically impossible to isolate a single wavelength, a very narrow band of wavelength is usually isolated. Two types of wavelength selectors are available, namely, filters and monochromators.

Filters: Filters operate by absorbing all but a restricted band of radiation from a source. A filter is characterised by its nominal wavelength, maximum percent band width and its effective bandwidth. (Figure 8.5)

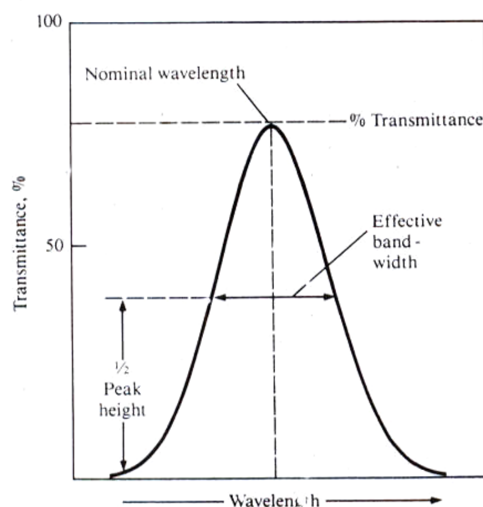


Figure 8.5: Output of a typical filter

Absorption filters are the most common type of filters. Absorption filter consists of a coloured glass piece that removes part of the incident radiation by absorption. Obviously, absorption filters are limited in application to the visible region.

Monochromators: Monochromators have mostly replaced filters in spectrometers due to their ability to produce very narrow band of wavelength over a considerable spectral range from radiation source. Such measurements are called spectral scanning. Monochromators as the name implies are capable of passing radiation of almost a single wavelength called monochromatic

radiation. The components of a monochromator are i) an entrance slit that provides a rectangular optical image of the radiation ii) a collimating lens or mirror which produces a parallel beam of radiation iii) a prism or grating that disperses the radiation into its component wave-lengths iv) a focusing element that reforms the image of the slit and focuses it on a planar surface called a focal plane and v) an exit slit that isolates the desired spectral band. The following figure shows the assembly for the two types of monochromators.

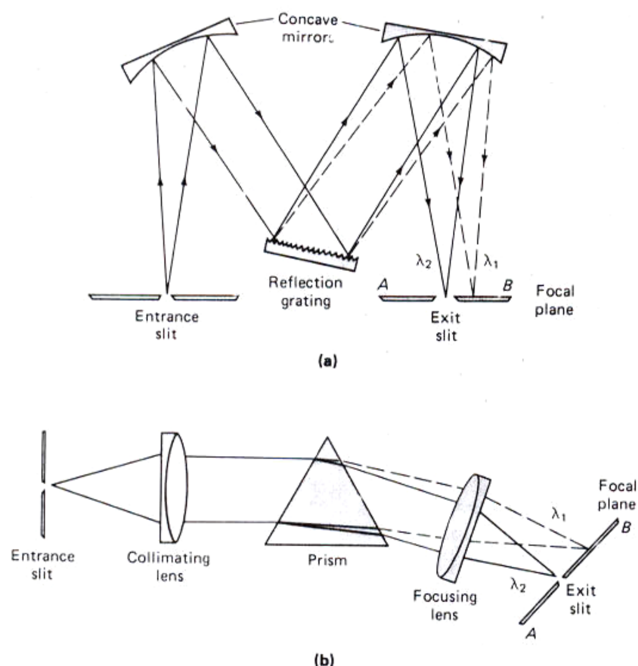


Figure 8.6: Two types of monochromators

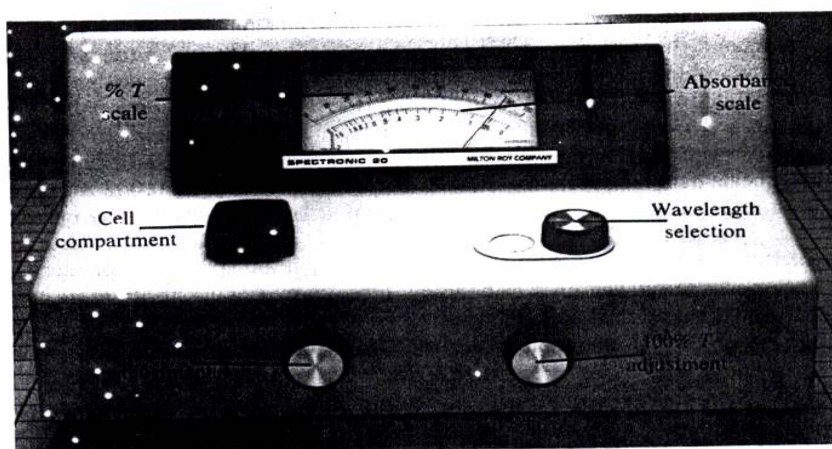
Sample holders: Sample holders are called cells or cuvets, which come in a variety of shapes and sizes appropriate for various instruments and experiments. The rectangular cell of 1 cm light path is the most widely used. Glass cells are used for measurements with visible radiation but cannot be used with ultra violet because of their strong absorption. Quartz or fussed silica cells, though more expensive are suitable for both UV and visible spectrometry.

The remaining two components of a spectroscope are the radiation detector and read-out device. The components of spectroscope described above have been assembled in various ways to produce a number of designs of instruments. Some are simple but other are very sophisticated. Therefore, their costs also vary widely. No single instrument is best for all purposes and selection must be based on the type of work for which the instrument is intended and by the economics of its application.

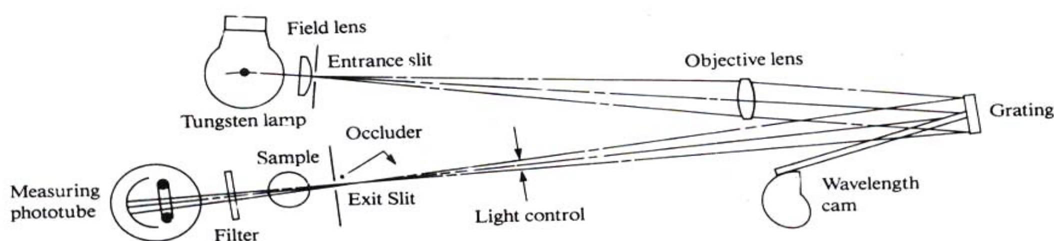
There are some common names for spectroscopic instruments. A **photometer** is a simple instrument that can be used for absorption, emission and fluorescence measurements with ultra violet, visible or infrared radiations. A photometer is distinguished by its use of absorption or interference filters for wavelength selection and a photoelectric device for measurement radiant power. Instruments used for absorption measurements with visible radiation are sometimes called **photoelectric colorimeter** or simply **colorimeter**. A photometer that is employed for fluorescence measurements exclusively is called a **fluorometer**. A spectrometer is a spectroscopic instrument equipped

with a monochromator. A spectrometer equipped with a photo transducer is called a **spectrophotometer**. Fluorescence spectrometers are often called **spectrofluorometers**.

Spectrophotometers: Numerous spectrophotometers are available to choose from. Some are designed for the visible region only, where as others are for both visible and ultraviolet regions. Spectrophotometers meant for the visible region are called **photoelectric colorimeters.** They can be used in the wavelength range of 380 to 800 nm. **Spectronic 20** is a typical commercial model of a photoelectric colorimeter (Figure 8.7).



(a)



(b)

Figure 8.7: a) Spectronic 20 spectrophotometer, b) its optical diagram

The instrument employs a tungsten filament lamp light source, and a reflection grating for wavelength selection. The selected wavelength of radiation is passes through the sample tube and to a phototube. The amplified electrical signal then powers a meter calibrated in % transmittance and absorbance.

Single and double beam spectrophotometers: Single beam instruments are simple and less expensive than double beam instruments. In this type of instruments, the cells containing reference and sample solutions have to be placed alternatively to tale measurements. Double beam spectrophotometers have two optical paths, which pass through the sample and reference solutions simultaneously. Such instruments also have provision for measurement of absorbance or transmittance of solutions continuously.

8.3.2 Atomic Spectroscopy

In the previous section, you have studied molecular absorption spectroscopy. Atomic spectroscopy is based on absorption, emission and fluorescence phenomena. Atomic spectroscopy is used for the qualitative and quantitative determination of more than 70 elements. Sensitivities of atomic methods are very high of the order of parts- per- million (ppm) to parts- per- billion (ppb). The reason that thermal emission methods are little used for determining

molecular species is that most molecules decompose at the temperatures required for producing atomic spectra.

Atomic absorption spectroscopic methods are characterised on the basis how the sample is atomised. The most convenient and common method followed is by using a suitable flame. Therefore, the instrumentation based on flame atomisation only will be discussed in this section. There are three methods under flame atomisation, viz. i) atomic absorption spectroscopy (AAS), ii) atomic emission spectroscopy (AES), and iii) atomic fluorescence spectroscopy (AFS). Among the three, AAS and AES have a lot of similarities, but it is important to know the differences.

Atomic Absorption and Emission Spectroscopy

Both AAS and AES are extremely important and valuable analytical techniques. It is well known that coloured radiations are produced when salts such as those of sodium, calcium, copper etc. are introduced into normal flames. Talburt investigated similar flame spectra of strontium, and lithium. In 1960, and 1961 Bunsen and Kirchoff discovered the elements caesium and rubidium by observing their flame spectra. It is the emission of such characteristic radiation and the determination of the intensity of this, which form the basis of atomic emission photometry. Atomic absorption spectroscopy deals with the external radiation absorbed by an element in a flame under certain conditions.

Flame atomisation: The critical component of a flame spectrometer is the flame atomiser. A flame atomiser consists of a pneumatic nebulizer, which converts the sample solution into a mist that is then fed into a burner. The most common type of nebulizer is based on aspiration in which the sample is sucked through a capillary tube by a high-pressure stream of gas flowing around the tip of the tube.

Burning a fuel with air or an oxidant like oxygen or nitrous oxide produces the flame in the burner. Air fuel mixtures produce flame having temperature in the range of 1700 to 2400°C, which is suitable for exciting only alkali and alkaline earth metals. For heavy metals, fuel- oxygen or nitrous oxide flames, which produce temperature in the range of 2500 to 3100 ^{0}C, are required.

Emission and absorption spectra for both atoms and elementary ions are obtained from the flame. When atoms and ions are heated in a flame, they are excited to various stages of energy levels. The excited species on relaxation produces the emission spectra and the unexcited species can absorb energy from an external source for producing absorption spectra. The external source for producing absorption spectra is usually a hollow-cathode.

Hollow-cathode lamp: The most useful radiation source for AAS is the hollow-cathode lamp. It consists of a tungsten anode and a cylindrical cathode sealed in a glass tube containing an inert gas such as argon. The cathode is made up of or coated with the metal to be analysed. Application of a high potential across the electrodes causes ionisation of the argon and generation of a current. The argon cations migrate towards the cathode and strike with sufficient energy to dislodge some of the metal atoms producing an atomic cloud. Some of the metal ions in the cloud in the excited state emit their characteristic wavelengths as they return to the ground state. The emitted radiation is allowed to be absorbed by the unexcited atoms/ions of the same species in the flame and the extent of absorption is measured. Hollow-cathode

lamp for a large number of elements is available. The development of the hollow-cathode is widely regarded as the single most important event in the evolution of atomic absorption spectroscopy. The remaining components of AAS or AES are similar to those present in molecular absorption spectrometers.

Check Your Progress Exercise 2

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. Describe the basic components of an absorption spectroscope?

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2. What is a monochromator?

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3. Differentiate between colorimeter and spectrophotometer.

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4. Describe a Hollow-cathode lamp.

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8.3.3 Refractometry

Refractometry involves the determination of the refractive index of a solution. It is applied to both qualitative and quantitative analysis such as identification of oils and fats, concentration of sugar solutions and total soluble solids content of fruit juices.

Refractive index is an important property of matter. You have already learnt about the propagation of light through vacuum and other media. Refractive index is defined as the ratio of the velocity of a radiation of a particular frequency in vacuum to the velocity in the medium under consideration. When a ray of light travels obliquely from one medium into another of different density, its direction is changed (bent) on passing through the interface between the two media. This phenomenon is called refraction. This phenomenon can be easily explained with the help of the following Figure 8.8.

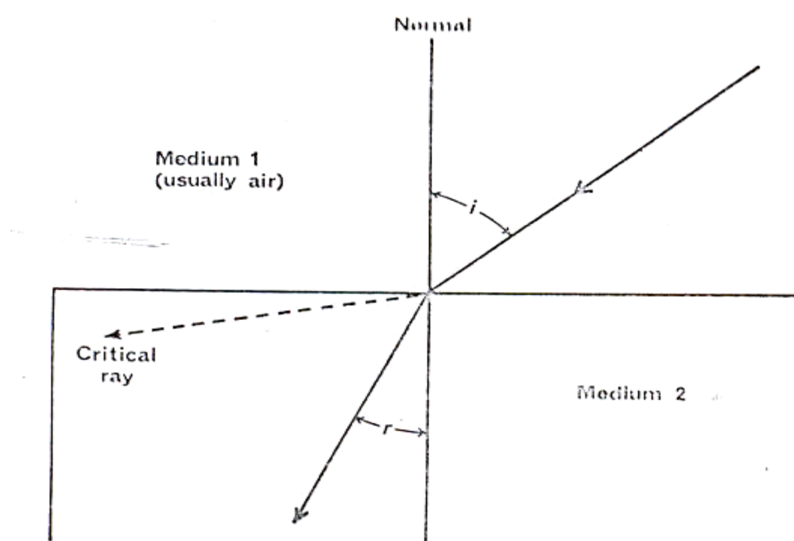

Figure 8.8: Refraction of light

The angle made by the incident ray in the first medium is called the angle of incidence, i and the corresponding angle in the second medium is termed the angle of refraction, r. sin i and sin r are directly proportional to the velocities of the radiation in the two media. Therefore, refractive index of the medium:

$$n = \frac{\sin i}{\sin r}$$

The refractive index of a medium is characteristic of that medium under constant temperature of measurement and the wavelength of the incident radiation. **Refractive index, n** of a medium is denoted by $\mathbf{n_D^{20}}$ meaning that it is measured using radiation of sodium D- line at 20°C. Water has a value of η_D^{20} of 1.3330. An instrument used to measure the refractive index (extent of bending of the radiation) of a medium is called a refractometer. The most important type of refractometer is the Abbe' refractometer.

Abbe' refractometer

A simplified diagram of the optical system of Abbe' refractometer is shown below (Figure 8.9).

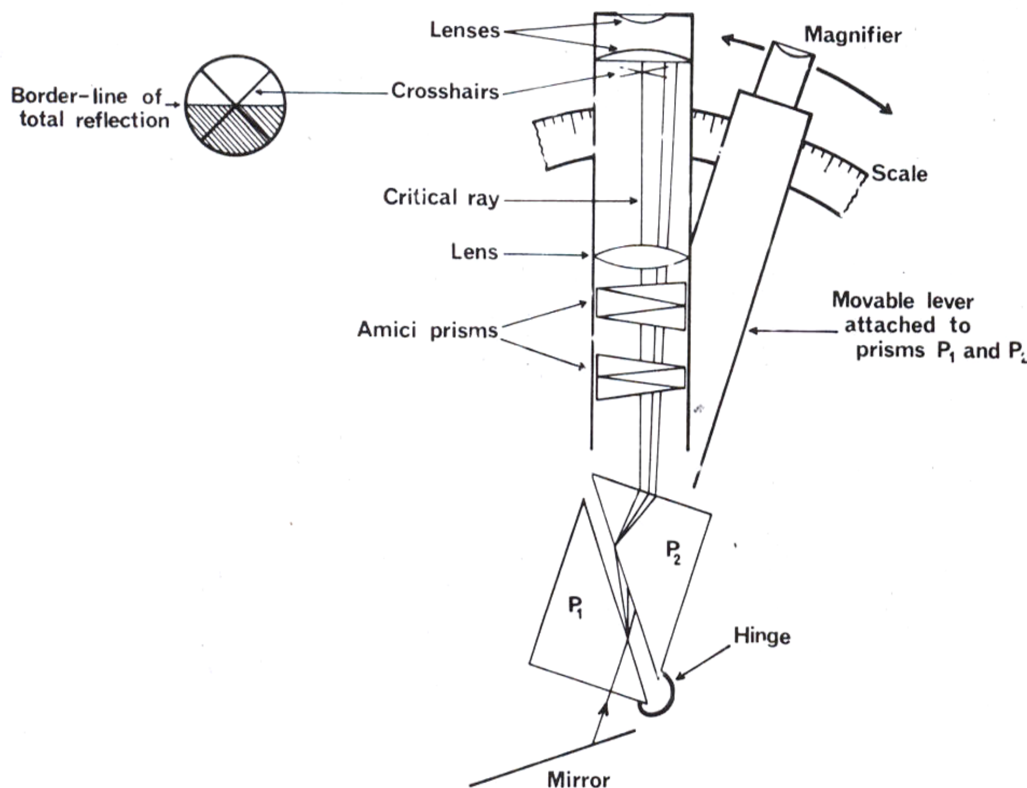

Figure 8.9: Abbe' refractometer

The most important component of the instrument is the double prism unit (P_1 and P_2). The unit can be rotated by means of a lever. At the other end, this lever moves over a fixed scale at which readings can be taken. The upper prism is made of a type of glass in which light has a velocity less than the velocity in any sample likely to be examined. The liquid sample is coated as a thin film between these two prisms. The upper prism is fixed while the lower one can be swung on a hinge to introduce sample and for cleaning.

White light entering the instrument is split by the two Amici prisms placed in the telescope of the instrument and only the sodium D-line is transmitted. Light falling on the polished surface of prism P_1 reflects an infinite number of rays through the liquid sample. The rays strike the surface of the upper polished prism, P_2 and are refracted. All light rays pass through the prism P_2, except those, which are parallel to the surface of the prism. The parallel rays are bent upwards and emerge from the prism at an angle, which is determined by the refractive index of the sample solution. The critical ray forms the border between light and dark portions of the field viewed by the observer. This border is aligned with the cross hairs of the eyepiece of the fixed telescope of the refractometer by slowly rotating the double prism unit. A fixed scale measures the angular relationship of the double prism to the telescope. Thus by moving the lever, the critical ray is scanned until it coincides with a fixed point in the instrument.

Usual Abbe' refractometer covers a range of refractive index either from 1.30 to 1.70 or from 1.45 to 1.84. The concentration of a known substance in a

solution can be readily determined by measuring the refractive index of the solution. This is the basis of °Brix (% of sugar) measurement. Abbe' refractometer provides separate scale based on the same principle.

8.3.4 Polarimetry

Polarimetry involves the measurement of the optical rotatory power of a substance. This method can also be used for both qualitative and quantitative analysis. You have learnt about the optical rotatory properties (levo and dextro) of compounds especially sugars. This is the basis of saccharimetry.

You have also learnt about plane polarisation of light. Many substances are capable of rotating the plane polarised light. Such substances are said to be optically active. Examples are amino acids, sugars, steroids, terpenes etc. The extent to which the plane-polarised light is rotated by an optically active compound is dependent on the concentration of its solution and path length under constant conditions of temperature, wavelength of the polarised light and solvent used. Thus the specific rotation of a solution is given by:

$$[\alpha]_{\lambda}^{T} = \frac{\alpha}{dc}$$

where, $[\alpha]_{\lambda}^{T}$ is the specific rotation at temperature T and λ wavelength of radiation. The angle of rotation measured is α and c is the concentration of the solution (in g/ml) contained in a sample tube d decimetres in length. Figure 8.10 shows a diagrammatic arrangement of a polarimeter.

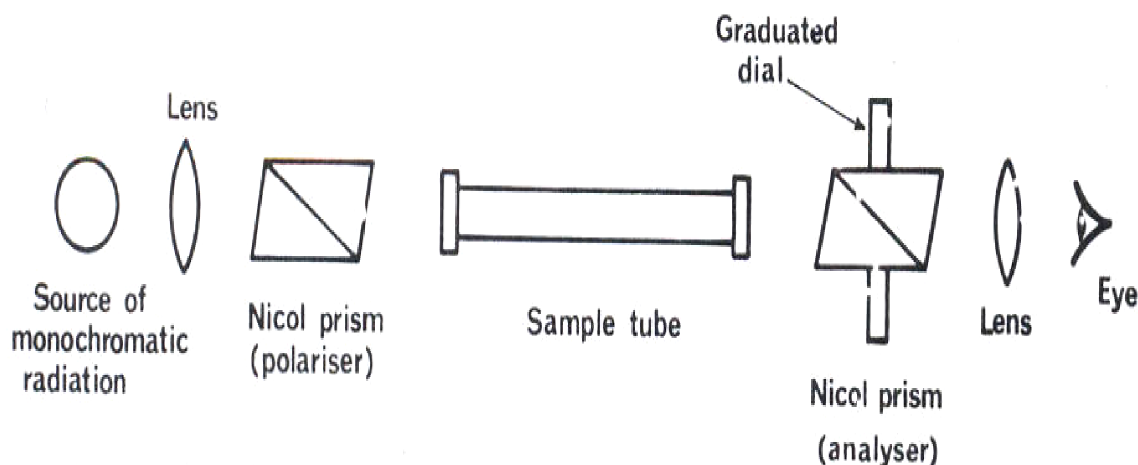

Figure 8.10: Components of a polarimeter

Monochromatic radiation from a source is passed through a Nicol prism, which polarises the radiation. Nicol prism is made of calcite or quartz crystal block cut diagonally in a special manner and cemented together. The polarised light passes through the sample when it is rotated. The analyser prism is another Nicol prism similar to the polariser prism. The prism is rotated to the extent of rotation of the radiation to allow it to be fully transmitted and observed by the human eye. The analyser is attached to a dial to indicate the number of degrees of rotation and direction of rotation.

Check Your Progress Exercise 3

Note: a) Use the space below for your answer.
b) Compare your answers with those given at the end of the unit.

1. What is meant by the refractive index of a medium?

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2. Explain the components of Abbe' refractometer and how it is used to measure the refractive index of a solution.

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3. What is polarisation of light?

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4. Describe the components of a polarimeter and explain how it is used to determine the concentration of an optically active solute.

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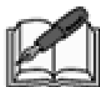


8.4 LET US SUM UP

A number of instruments based on the interactions of electromagnetic radiations with food components are used for analysis and quality control. These interactions could be in the form of absorbance, emission, refraction, and rotation of radiation. Among the instruments, those based on absorption of radiation by solutions are widely used for qualitative and quantitative analysis of elements and compounds. While colorimeters are used in the visible range of radiation, spectrophotometers can be used with ultra violet, visible and infrared regions.

Atomic absorption and emission spectroscopy find application in the determination of elements, especially metal ions.

Refractometers are based on the principle of refraction of light radiation. Refractometer is a handy instrument for measuring the total soluble solids (^{o}Brix) of fruit products and sugar solutions.

Polarimeter is based on the principle of rotation of plane polarised light by a solution of optically active substance. A number of food components, especially oils, and sugars exhibit optical activity. Therefore, they can be easily determined using a polarimeter.

8.5 KEY WORDS

Wavelength : Distance between two successive waves.

Frequency : Number of waves passing a fixed point per unit time.

Beer's law : Relationship between amount of radiation absorbed and the concentration of solute in the solution.

Monochromator : An optical instrument to produce a narrow band of radiation.

8.6 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   - Distance between two successive wave maxima or minima
   - Number of waves passing a fixed point per unit time

2. Your answer should include the following points:

   - Study of the interactions of electromagnetic radiations with matter
   - Absorption spectroscopy
   - Emission spectroscopy
   - Fluorescence spectroscopy

3. Your answer should include the following points:
   - Absorption of radiation by molecules
   - Absorption of radiation by atoms and ions
4. Your answer should include the following points:
   - Relationship between amount of radiation absorbed and the amount of absorbing substance.
5. Your answer should include the following points:
   - **Hints:** Use formula A = Ebc, Ans.33600

Check Your Progress Exercise 2

1. Your answer should include the following points:
   - Source of radiant energy
   - Wavelength selector
   - Sample holders
   - Radiation detector
2. Your answer should include the following points:
   - Instrument to produce monochromatic radiation
   - Prism or grating
3. Your answer should include the following points:
   - Absorption measurement with visible radiation
   - UV, visible and IR
4. Your answer should include the following points:
   - Emits characteristic radiations of metals
   - Atomic absorption spectroscope

Check Your Progress Exercise 3

1. Your answer should include the following points:
   - Ratio of velocity of radiation in vacuum to the velocity in the medium
   - Ratio of sin i to sin r
2. Your answer should include the following points:
   - Double prism unit
   - Amici prism and telescope
3. Your answer should include the following points:
   - Restricting the oscillations to one plane
   - Optical activity
4. Your answer should include the following points:
   - Nicol prism
   - Sample tube
   - Measuring scale

8.7 SOME USEFUL BOOKS

1. Hagris, L.G. (1988) Analytical Chemistry, Prentice Hall, New Jersey.
2. Mac Leod, A.J. (1973) Instrumental Methods of Food Analysis, Elek Science, London.
3. Skoog, D.A., and Leary, J.J. (1992) Principles of Instrumental Analysis, Saunders College Publishing, Florida.

EXPERIMENT 1 DETERMINATION OF ASCORBIC ACID BY TITRIMETRIC AND COLORIMETRIC METHODS

Structure

1.1 Introduction
Objectives
1.2 Experiment 1a: Dye Titration Method
Principle
Requirements
Procedure
Observations
Calculations
Result
1.3 Experiment 1b: Xylene Extraction and Colorimetric Method
Principle
Requirements
Procedure
Result
1.4 Precautions

1.1 INTRODUCTION

Fruits and vegetables are important sources of ascorbic acid (vitamin C). Ascorbic acid being unstable under different storage and processing conditions, it is important to know its residual content in food products. The most satisfactory chemical method of estimation is based on the reduction of 2,6-dichlorophenol indophenol by ascorbic acid. This can be performed either by titration or by colorimetric method. In this experiment you will be learning both the methods.

Objectives

After studying and performing this experiment, you should be able to

- prepare different types of food samples for ascorbic acid estimation;
- determine the ascorbic acid content by dye titration method; and
- determine the ascorbic acid content by xylene extraction and colorimetric method.

1.2 EXPERIMENT 1a: DYE TITRATION METHOD

1.2.1 Principle

2,6-dichlorophenol indophenol dye, which is blue in alkaline solution and red in acid solution, is reduced by ascorbic acid to a colourless form. The reaction is quantitative and can be performed by titration. This reaction is practically specific for ascorbic acid in fresh fruits and vegetables. Sulphur dioxide

present in products like squashes can deduce the dye and thus interferes in the estimation. Condensing SO_2 with formaldehyde can eliminate this interference.

1.2.2 Requirements

Apparatus/Glassware

Microburette, 10 ml capacity with 0.05 ml sub-graduations

Burette stand

Volumetric flask, 100 ml

Pipette, 1 ml

Conical flask, 100 ml

Analytical balance, 0.1 mg sensitivity

Whatman No.1 filter paper circles

Glass funnel, 2” dia.

Chemicals and Reagents

i) 3% (w/v) Metaphosphoric acid (HPO_3): Prepare by dissolving the sticks or pellets of HPO_3 in distilled water.

ii) Ascorbic acid standard: Weigh accurately 100 mg of L-ascorbic acid and make up to 100 ml with 3% HPO_3 solution. Dilute 5 ml to 50 ml with 3% HPO_3 solution (1 ml = 0.1 mg of ascorbic acid).

iii) Dye solution: Dissolve 50 mg of the sodium salt of 2,6-dichlorophenol indophenol in approximately 150 ml of hot distilled water containing 42 mg of sodium bicarbonate. Cool, filter and dilute with distilled water to 200 ml. Store in a refrigerator and standardize every day.

iv) Formaldehyde, 40% solution.

v) Conc. Hydrochloric acid.

1.2.3 Procedure

Standardization of Dye

Pipette out 5 ml of the standard ascorbic acid solution into a 100 ml conical flask and add 5 ml of the 3% HPO_3 solution. Fill the microburette with the dye solution. Titrate the ascorbic acid solution with the dye solution to a pink colour, which should persist for 15 sec. Note the Titre value. Calculate the dye factor.

Volume of ascorbic acid solution taken for titration = 5 ml

Volume of dye solution required (titre) = V = ----- ml

Dye factor = mg of ascorbic acid per ml of the dye

Since 5 ml of the standard ascorbic acid solution contains 0.5 mg ascorbic acid:

$$\text{Dye factor} = \frac{0.5}{\text{Titre}} = \frac{0.5}{V} = \text{mg ascorbic acid per ml dye}$$

Preparation of Sample

Juices and liquid products: Take 10-20 g sample and make up to 100 ml in a volumetric flask with 3% HPO_3 solution. Filter through a Whatman No. 1 filter paper.

Solid or semi-solid products: Blend 10-20 g sample with 3% HPO_3 solution and make up to 100 ml in a volumetric flask with 3% HPO_3 solution. Filter through a Whatman No. 1 filter paper.

Titration

Pipette out 2-10 ml of the sample extract into a 100 ml conical flask and titrate against the dye solution as above. The volume of the sample should be such that the tire value is in the range of 3-5 ml.

If the sample contains sulphur dioxide, to the pipetted out sample extract add 1 ml of the formaldehyde solution and 0.1 ml HCl, keep for 10 min and perform the titration.

1.2.4 Observations

Weight of sample taken for extraction with HPO_3 = W = ----- g

Volume of the sample made up with HPO_3 solution = 100 = ----- ml

Volume of sample extract taken for dye titration = V_1 = ----- ml

Volume of dye required (titre) = V_2 = ----- ml

1.2.5 Calculations

Ascorbic acid in V_1 ml of the sample extract = dye factor x V_2 = mg

$$\text{Therefore, ascorbic acid in 100 ml of the extract} = \frac{\text{Dye factor} \times V_2 \times 100}{V_1} = \text{mg}$$

Since W g sample was made up to 100 ml, ascorbic acid content of the sample (mg per 100 g)

$$= \frac{\text{Dye factor} \times V_2 \times 100 \times 100}{V_1 \times W} = \frac{\text{Dye factor} \times V_2 \times 10{,}000}{V_1 \times W}$$

1.2.6 Results

Ascorbic acid content of the sample = mg per 100 g.

1.3 EXPERIMENT 1b: XYLENE EXTRACTION METHOD

1.3.1 Principle

This method is based on measurement of the extent to which a 2,6-dichlorophenol indophenol solution is decolourised by ascorbic acid in sample

extracts and in standard ascorbic acid solutions. The excess dye is taken up in xylene and colour measured in a colorimeter at 520 nm. This method is particularly suitable for stored products in which considerable interfering substances are present.

1.3.2 Requirements

Colorimeter with sufficient number of sample tubes

Analytical balance, 0.1 mg sensitivity

Volumetric flask, 100 ml, and 1000 ml

Pipette, 10 ml

Conical flasks, 50 ml glass stoppered

Funnel

Whatman No.1 filter circles

Reagents

i) Acetate buffer- pH 4: Mix 500 ml of 50% sodium acetate ($CH_3COONa.3H_2O$) with 500 ml of glacial acetic acid.

ii) Dye: Dissolve 125 mg of 2,6-dichlorophenol indophenol (sodium salt) in warm distilled water, cool , make up to 100 ml in a volumetric flask and filter (stock solution). Dilute 18 ml to 100 ml with water. 1 ml of this solution should be equal to 0.1 mg of ascorbic acid. The stock solution of the dye may be stored in a refrigerator for a week.

iii) Meta phosphoric acid solution (3%): Dissolve 15 g of sticks or pellets of HPO_3 in distilled water and dilute to 500 ml.

iv) Standard ascorbic acid solution: Weigh exactly 100 mg of ascorbic acid and make up to 100 ml with 3% HPO_3 solution. Dilute 10 ml to 100 ml (1 ml = 0.1 mg ascorbic acid).

v) Xylene.

vi) Formaldehyde 40%.

vii) Anhydrous sodium sulphate.

1.3.3 Procedure

Sample extraction procedure followed for the titration method may be followed for this method also.

Standard Curve

Pipette out 0.0, 0.50, 0.75, 1.0, 1.5 and 2.0 ml of the standard ascorbic acid solution into 50 ml stoppered conical flasks. Make up the total volume in each flask to 2 ml with 3% HPO_3 solution. Add 1 ml water, 2 ml acetate buffer, 3 ml dye solution and 15 ml xylene in rapid succession. Stopper the conical flasks and shake vigorously for 10 sec to extract the excess dye into the xylene. Allow the layers to separate. With a pipette completely draw out the water layer below the xylene layer and discard. Add a small quantity (0.5-1 g) of anhydrous Na_2SO_4 to the xylene layer to remove traces of moisture. Transfer

the xylene extracts to the colorimeter tubes and measure the absorbance at 520 nm. Set the instrument to 100% transmittance using xylene as blank. Plot the absorbance values (A) against ascorbic acid (mg) on a graph paper to get the standard curve. You will see that as the concentration of ascorbic acid in the reaction mixture increases, the absorbance value decreases.

Vol. of ascorbic acid (ml)	Ascorbic acid (mg)	Absorbance (A)
0.0	0.00	
0.5	0.05	
0.75	0.075	
1.0	0.10	
1.5	0.15	
2.0	0.20	

Sample

Take 2 ml sample extract in a stoppered conical flask, add 2 ml of buffer, 1 ml of 40% formaldehyde and mix. Allow to stand for 10 min. Then add 3 ml dye solution, stopper and shake for 10-15 sec. Follow the remaining steps as done in the case of standard curve preparation. From the standard curve note the ascorbic acid content (mg) in the 2 ml sample extract taken for the estimation.

1.3.4 Observations

Absorbance of the xylene extract = A_1

Corresponding ascorbic acid content from the standard curve = W_1 = ---- mg

Weight of the sample taken for extraction = W_2 = ----- g

Volume of the sample made up for ascorbic acid extraction = 100 = ---- ml

1.3.5 Calculations

From the data, W_1 mg of ascorbic acid is present in 2 ml of the sample extract.

As W g of the sample was made up to 100 ml for extraction of ascorbic acid, the ascorbic acid content of the sample (mg per 100 g)

$$= \frac{W_1 \times 100 \times 100}{2 \times W_2} = \frac{W_1 \times 5000}{W_2}$$

1.3.6 Results

Ascorbic acid of the sample = mg per 100 g.

1.4 PRECAUTIONS

The general precautions mentioned in the course ‘Introduction’ and those indicated in the experiments should be followed meticulously.

The colorimeter and the sample tubes should be handled with care.

EXPERIMENT 10 SENSORY EVALUATION OF FOOD PRODUCTS – HEDONIC RATING TEST

Structure

10.1 Introduction
Objectives
10.2 Experiment: Hedonic Rating Test for Ketchup Samples
Principle
Requirements
Procedure
Observations and Calculations
Result
10.3 Precautions

10.1 INTRODUCTION

Sensory quality of food products is of great importance to both the producer or processor and the consumer. Good quality products attract the consumer by satisfying his aesthetic and gustatory senses. Therefore, it is always the endeavour of the processor to produce the best quality product or produce a product having certain qualities accepted by the consumer in a product already available in the market.

You have already learnt the importance and various aspects of sensory analysis in the theory unit 7.2.4 "Sensory analysis of foods/ beverages". Please brush up before performing this test. There are different sensory test methods to suit specific purposes. One of the frequent requirements of the processor while developing a product is to find out the relative acceptability of his product compared to three or four market sample or to develop a product close to the best in the market. One of the simplest sensory test methods to generate such information is the Hedonic rating test. In this exercise you will learn the method and carry out the test.

Objectives

After studying and performing this experiment, you should be able to

- carry out Hedonic rating test on a set of food products; and
- analyse the data to find out the order of their quality of consumer preference for the samples.

10.2 EXPERIMENT: HEDONIC RATING TEST FOR KETCHUP SAMPLES

10.2.1 Principle

The Hedonic rating test is used to measure the consumer acceptability and preference of food products. The panellist is asked to rate the acceptability of the product on a scale of 9 points, ranging from "like extremely" to "dislike extremely". The data are analysed to find out the average of the panellists ratings from which the order of preference if found out.

10.2.2 Requirements

The primary requirement for any sensory test is the panel of members (panellists). For many sensory tests including the Hedonic rating test, a semi-trained panel is sufficient. The minimum number of panellists required for this test is ten. They should be selected from a larger number of people and should be familiarised (trained) with the quality attributes of the product being tested (or they should be familiarised by proper briefing) and the procedure. They should also have at least average sensitivity to the sensory quality attributes like colour and appearance, flavour and taste etc. Besides, they should be willing to spend the time to do the test.

The other requirements are a well-lighted (white light) room with tables (preferably white tops) and chairs. Assuming that 4 samples (tomato ketchup as in this test) are evaluated and 10 panellists are going to evaluate the products, the following glassware and other items are also required.

White porcelain saucers : 12 (These are sufficient for serving a set of the four samples to three panellists. The saucers can be cleaned and reused)

Teaspoons : 12

Glass tumblers for water : 10

Bread : 1 loaf

Evaluation cards (Sample shown below) : 10

Specimen evaluation card

HEDONIC RATING TEST

Name…………………… Date…….......

Product: Tomato ketchup

Taste the four samples of tomato ketchup and check how much you like or dislike each one. Use the appropriate scale to show your attitude by checking at the point that describes your feeling about the sample.

Scale	**Ketchup samples**			
	Code No.	**Code No.**	**Code No.**	**Code No.**
Like extremely				

Like very much				
Like moderately				
Like slightly				
Neither like nor dislike				
Dislike slightly				
Dislike moderately				
Dislike very much				
Dislike extremely				
Reason for like/ dislike				

(Signature of panellist)

Three columns are provided to accommodate more than one sample if all of them fall at the same point.

10.2.3 Procedure

In this test, four tomato ketchup samples are tested for the preference. One sample could be the one prepared in a factory and the others are the three market samples. Otherwise, all the four samples could be market samples. The ketchup bottles are first marked with code numbers. It is preferable to have three digit random number codes to avoid bias. Single digit numbers like 1,2,3, 4 are likely to cause bias. For example, some panellists may have a tendency to assume No. 1 is the best and No.4 is the worst and vive versa. Random numbers can be taken from random number tables or generated from a calculator. An example of assigning random numbers to four samples is shown below.

Ketchup sample	Code No.
A	897
B	281
C	951
D	418

In the same way, the sets of four saucers are also numbered and small quantities of the ketchup samples are taken in them. Before starting the evaluation, the panellists are briefed about the test procedure, what to look for in the quality of tomato ketchup, two or more samples may be given the same rating if found so etc. Each product has its own quality attributes. For example, in the case of tomato ketchup, good quality attributes are bright characteristic tomato red colour, thick consistency, does not flow easily when the saucer is tilted, does not show separation of serum around the ketchup sample in the saucer etc.

The set of four saucers containing the samples is given to each panellist along with the evaluation card. Drinking water to rinse mouth in between tasting of two samples to clear the taste of the previous sample is provided. Similarly, cubes of bread are provided for eating for the same purpose. If a panellist requires more samples, the same should be provided. On completion of the evaluation, the evaluation cards are collected and the data are analysed as shown below.

10.2.4 Observations and Calculations

To analyse the results, numerical values are assigned to each point on the scale, 1 is usually given to 'like extremely' and 9 to 'dislike extremely'. The scores received for each sample from all the panellists are averaged and compared. The data are tabulated as shown in the following table. The numerical values given in the table are only examples. You have to enter the actual values.

Panellist	Sample-A	Sample-B	Sample-C	Sample-D
1	3	3	6	5
2	4	3	4	5
3	3	1	4	4
4	2	2	3	4
5	3	1	2	3
6	3	2	7	6
7	2	2	3	5
8	3	2	3	8
9	5	3	7	7
10	3	2	4	6
Total	31	21	43	53
Mean score	3.1	2.1	4.3	5.3

10.2.5 Results

Since the numerical scores are assigned in the reverse order i.e., 1 for the highest quality and 9 for the lowest quality point, lower the score total or score average higher the preference. Therefore, the rating of the quality (preference) of the four tomato ketchup samples is in the following order:

Sample-B > Sample-A > Sample-C > Sample-D

10.3 PRECAUTIONS

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.

EXPERIMENT 2 DETERMINATION OF SODIUM CHLORIDE

Structure

2.1 Introduction
 Objectives
2.2 Experiment
 Principle
 Requirements
 Procedure
 Observations
 Calculations
 Result
2.3 Precautions

2.1 INTRODUCTION

Salt (sodium chloride) is an important ingredient in several food products like pickles, chutneys, and sauces. Brine (dilute salt solution) is the common covering liquid for most of the low acid canned products like vegetables. In products like "pickle in brine", the minimum salt content has been specified.

The approximate salt content in brine solutions can be measured using a salinometer (hygrometer). However, for more accurate determination of sodium chloride, silver nitrate titration method is mostly followed.

Objectives

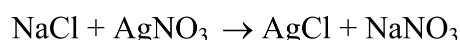
After studying and performing this experiment, you should be able to

- determine the salt content of food products.

2.2 EXPERIMENT

2.2.1 Principle

When a sample extract containing sodium chloride to which a few drops of potassium chromate solution is added, is titrated with standard silver nitrate solution, silver nitrate precipitates chloride as silver chloride. Immediately on completion of the precipitation reaction, the excess of silver nitrate reacts with potassium chromate forming reddish brown silver chromate, which is the end point. The quantity of silver nitrate used for the precipitation is the measure of the sodium chloride content of the sample.



$$NaCl + AgNO_3 \rightarrow AgCl + NaNO_3$$

2.2.2 Requirements

Glassware and other items

Chemical balance

Burette, 25 ml −1

Conical flask, 250 ml −2

Measuring cylinder

Whatman No.1 filter paper circles,

Funnel, 4 inch –2

Reagents

5% Potassium chromate solution (indicator)

0.1N Silver nitrate solution

Calcium carbonate powder

2.2.3 Procedure

Weigh 25 to 50 g of homogenized sample depending on the salt content. Dilute with distilled water and neutralize with 0.1N sodium hydroxide solution using phenolphthalein as indicator. Transfer to a 250 ml volumetric flask, make up to volume, shake and filter. Titrate an aliquot with 0.1 N silver nitrate solution adding about 1ml of 5% aqueous potassium chromate solution as indicator. Note the volume of silver nitrate solution required to produce the reddish brown end point colour. Carry out a blank titration with distilled water of same volume as the sample aliquot.

2.2.4 Observations

Weight of the sample	= W = ------- g
Volume made up	= V = ------- ml
Volume taken for titration	= V_1= ------- ml
Volume of silver nitrate solution required for sample	= V_2 = ------ ml
Volume of silver nitrate required for blank titration	= V_3= ------- ml
Normality of the $AgNO_3$ solution	= N

2.2.5 Calculations

1000 ml 1 N $AgNO_3$ solution = 1 g mole of sodium chloride = 58.45 g.

Therefore, % NaCl in the sample =

$$\frac{58.45\ (\text{sample titre} - \text{blank titre})\ N \times \text{volume made up} \times 100}{1000 \times \text{aliquot volume taken for titration} \times \text{weight of sample}}$$

i.e. % sodium chloride in the sample = $$\frac{58.45\ (V_2 - V_3)\ N \times V}{10 \times V_1 \times W}$$

2.2.6 Results

Salt content in the sample = Percent.

2.3 PRECAUTIONS

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.

Handle silver nitrate and its solution with care. They can leave permanent stain on the skin and cloth.

EXPERIMENT 3 DETERMINATION OF TOTAL CAROTENOIDS AND BETA-CAROTENE BY COLORIMETRIC METHOD

Structure

3.1 Introduction
Objectives
3.2 Experiment: Total Carotenoids and Beta-carotene Estimation
Principle
Requirements
Procedure
Observations
Calculations
Result
3.3 Precautions

3.1 INTRODUCTION

Carotenoids are a group of yellow, orange and orange-red fat-soluble pigments widely distributed in nature. The carotenoids are of great nutritional importance as some of them are converted into vitamin A. Several fruits and vegetables especially the leafy vegetables are good sources for carotenoids. Therefore, estimation of their concentration in fresh and processed foods is important.

Extracting into solvents like petroleum ether and measuring the colour at 452 nm in a colorimeter most commonly estimate total carotenoids in food materials. Beta-carotene is chromatographically separated from total carotenoids on a suitable adsorbent and colorimetrically estimated.

Objectives

After studying and performing this experiment, you should be able to

- extract carotenoid pigments from food materials;
- separate beta-carotene from the total carotenoids chromatographically; and
- estimate them colorimetrically.

3.2 EXPERIMENT: TOTAL CAROTENOIDS AND BETA-CAROTENE ESTIMATION

3.2.1 Principle

Carotenoid pigments (carotenes, xanthophylls and santhophyllesters) being fat-soluble substances, can be extracted into water immiscible solvents like petroleum ether. The absorbance of the extract is measured in a colorimeter or spectrophotometer, and the carotenoids concentration is calculated using a standard curve. Beta-carotene can be separated from total carotenoids extract chromatographically on a magnesium oxide- supercel column and separately estimated.

3.2.2 Requirements

Apparatus

Colorimeter or specrophotometer

Chromatographic column, 150 x 19 mm (ID) glass tubes with constriction at one end to attach 3 mm glass tubing. The column should be fixed to a rubber cork, which should fix to a 100 ml Buchner flask.

Plunger for the preparation of the adsorption column.

Buchner flask, 100 ml –2

Suction pump

Analytical balance, 0.1 mg sensitivity

Pestle and mortar

Volumetric flask, 100 ml –6

------ do ----- , 250 ml –1

------- do ---------, 25 ml –2

Pipettes, 5 and 10 ml

Conical flask, 250 ml –2

Funnels, 3 inch –2

Separating funnel, 250 ml –2

Reagents

Petroleun ether (b.p. 65-70^{0}C)

Acetone

Chloroform

Anhydrous sodium sulphate

Adsorbent: Mix one part of magnesium oxide (MgO) with three parts of supercel.

3% acetone in petroleum ether

Sea sand, purified

Surgical cotton

3.2.3 Procedure

Standard Curve of β-carotene

Weigh accurately 25 mg of β-carotene and dissolve in 2.5 ml chloroform and make up to 250 ml with petroleum ether (1 ml = 0.1 mg or 100 μg). Dilute 10 ml of this solution to 100 ml with petroleum ether in a volumetric flask (1 ml = 10 μg). Pipette 5, 10, 15, 20, 25 and 30 ml of this solution to separate 100 ml volumetric flasks, each containing 3 ml acetone and dilute to mark with petroleum ether. The concentration of β -carotene in these solutions will be 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 μg per ml. Measure the absorbance of the solutions at

452 nm using 3% acetone in petroleum ether as blank. Draw a graph by plotting absorbance against concentration. Record the data as follows.

β-carotene (μg/ ml)	Absorbance(A)
0.5	
1.0	
1.5	
2.0	
2.5	
3.0	

Sample Extraction

Weigh a well-blended sample (5 to 25 g) containing 10 to 500 μg total carotenoids. Grind in a pestle and mortar with acetone adding small quantity of pure sand. Filter through cotton into a conical flask. Continue extraction and filtration till the residue is colourless. Transfer the combined filtrate to a separating funnel. Add 10 to 15 ml petroleum ether followed by distilled water to transfer the pigments to the petroleum ether phase. Drain out the aqueous phase and filter the petroleum ether extract through anhydrous sodium sulphate. Make up the petroleum volume of the ether extract to 25 ml in a volumetric flask with petroleum ether. Measure the absorption of the solution at 452 nm. Calculate the total carotenoids contents using the standard curve. The results are expressed in terms of β-carotene as μg per 100 g of the material.

Chromatographic Separation of β-carotene

Preparation of column: Attach the column to a Buchner flask, apply vacuum and pack the glass column tightly with the adsorbent to a height of about 10 cm. alternatively, press the adsorbent, 2-3 times with a plunger to ensure a tight column. Add anhydrous Na_2SO_4 to the top of the column to about 1 cm height.

Sample adsorption and elution: Wash the column with 25 to 50 ml petroleum ether with suction. When the petroleum layer has almost reached the Na_2SO_4 surface, disconnect suction pump and attach the column tube to another clean and dry Buchner flask. Pipette out 5 to10 ml of the sample extract into the column and apply suction. Wash the column continuously with 3% acetone in petroleum ether (eluent) taking care not to allow the solvent layer to go below the Na_2SO_4 layer. β-carotene moves out of the column prior to all other pigments. When the β-carotene band has flowed out completely, disconnect suction and transfer the contents of the Buchner flask to a volumetric flask and make up to volume with the eluent. Measure the absorbance of the solution at 452 nm using 3% acetone in petroleum ether as blank.

3.2.4 Observations

Weight of sample taken for carotenoids extraction = W = ----- g

Volume of the petroleum ether extract of the sample = V = ----- ml

Absorbance of the solution = A

Concentration of carotenoids in the solution (from std. curve) = C = μg/ml

Volume of the petroleum ether extract taken for

Chromatography = V_1 = ----- ml

Volume of the β- carotene band made up to = V_2 = ----- ml

Absorbance of the β-carotene extract = A_1

Concentration of β-carotene in the solution (from std. curve) = C_1 = -- μg/ml

3.2.5 Calculations

Total Carotenoids

Concentration total carotenoids in the petroleum ether extract = C = μg/ ml

Therefore, total carotenoids content in V ml of the petroleum ether extract

$$= C \times V = \mu g$$

$C \times V$ μg carotenoids are present in W g of the sample

Therefore, total carotenoids content in the sample = $\frac{C \times V \times 100}{W}$ = μg per 100 g

β-carotene

Concentration of β-carotene in the β-carotene eluate = C_1 = μg/ml

Therefore, β-carotene content in V_2 ml of the eluate = $C_1 \times V_2$ = μg

$C_1 \times V_2$ μg of β-carotene is present in V_1 ml of the extract taken for chromatography.

Therefore, β-carotene content in the sample = $\frac{C_1 \times V_2 \times V \times 100}{V_1 \times W}$ = μg per 100 g

3.2.6 Results

Total carotenoids content of the sample = μg per 100 g.

β-carotene content of the sample = μg per 100 g.

3.3 PRECAUTIONS

β-carotene is unstable to light and susceptible to air-oxidation. Therefore, the sample extracts should be prevented from oxidation and light.

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.

Never handle petroleum ether near a flame. The solvents should be handled only in a well-ventilated room or inside a hood with exhaust. Avoid inhaling the solvents directly.

EXPERIMENT 4 DETERMINATION OF SULPHUR DIOXIDE

Structure

4.1 Introduction
Objectives
4.2 Experiment
Principle
Requirements
Procedure
Observations
Calculations
Result
4.3 Precautions

4.1 INTRODUCTION

Sulphur dioxide and sulphites are versatile food preservatives having several beneficial functions. Sulphur oxide added to food products as preservative may exist as undissociated sulphurous acid, as free bisulphite ion, as free sulphite ion, and/or as combined SO_2 in the form of hydroxy sulphonates. However, they can cause harmful effects if consumed in higher quantities. Therefore, like for all other preservatives, maximum permissible limits of sulphites in foods have been laid down. Besides, sulphites are not permitted in all foods.

There are two methods used for the estimation of sulphites in foods. Both of them make use of the reducing property of sulphur dioxide. In one method, iodine is used to oxidize sulphur dioxide (sulphurous acid to sulphuric acid in aqueous solution) and in the other method, hydrogen peroxide is used for the oxidation reaction after liberating sulphur dioxide from the product. The latter method is more reliable and hence followed widely.

Objectives

After studying and performing this experiment, you should be able to

- estimate the sulphur dioxide content of food products by the distillation method.

4.2 EXPERIMENT

4.2.1 Principle

This method measures total sulphur dioxide in food products. Sulphites present in the product are liberated as sulphur dioxide by boiling with hydrochloric acid. The liberated sulphur dioxide is absorbed in hydrogen peroxide solution, which oxidizes it to sulphuric acid. Sulfite content is directly related to generated suphuric acid, which is determined by titration with standard sodium hydroxide solution.

The reactions involved are:

$$SO_2 + H_2O \rightarrow H_2SO_3$$

$$H_2O_2 + SO_2 \rightarrow H_2SO_4$$

4.2.2 Requirements

Apparatus

a) All glass distillation apparatus for determination of sulphur dioxide shown in the diagram below.

Diagram: SR p 307

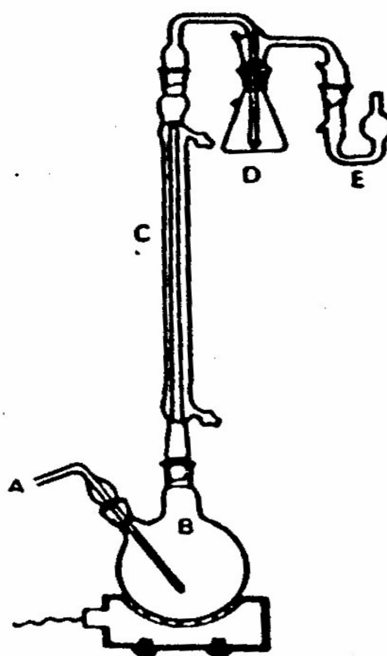


Figure 4.1: All glass distillation apparatus for determination of sulphur dioxide. A) glass inlet tube, B) 500ml round-bottomed flask, C) condenser, D) 250 ml conical flask, E) trap

b) Burette: - 10 ml

Reagents

a) **Aqueous hydrochloric acid:** -4M. For each analysis, prepare 90 ml solution by adding 30 ml HCl to 60 ml deionized water.

b) **Methyl red indicator: -**Dissolve 250 mg methyl red in 100 ml ethanol.

c) **0.05 N NaOH solution.**

d) **3% Hydrogen peroxide solution: -**. For each analysis, dilute 3 ml reagent grade 30% H_2O_2 to 30 ml with distilled water. Just prior to use, add 3 drops methyl red indicator and titrate with 0.01N NaOH to yellow end point.

e) **Nitrogen gas: -**High purity, used with regulator to maintain flow of 200 ml/min.

4.2.3 Procedure

Circulate cold water through condenser of the distillation apparatus. Add from a graduated cylinder, 20ml of 3% hydrogen peroxide solution to the conical flask (D) and 5 ml to the trap (E). Assemble the apparatus and connect condenser. Weigh 50g of blended sample into the round-bottomed flask (B) through gas inlet tube joint, using 300ml of water. Replace the inlet tube immediately, making sure all connections are well greased and tight. Remove the inlet tube, and slowly add 20ml of 4N HCl. Ensure that bubbles of nitrogen gas enter the receiving flask through the gas inlet tube. If not, check joints for leaks. Adjust nitrogen to give a flow of 15 to 20 bubbles per minute through the tube. Heat the content of the flask to boil and adjust the heater to give a slow boil. Continue boiling for 30 mins. Stop heating and disconnect the assembly and remove the conical flask and the trap containing hydrogen peroxide. Transfer the hydrogen peroxide solution from the trap into the conical flask and rinse the trap with water and transfer the rinsing to the flask.

Determination

Add 3 drops of the indicator. Immediately titrate contents of conical flask (D) with 0.05N NaOH to yellow end point that persists for about 20 seconds. Compute sulfite content, expressed in mg SO_2/Kg food (ppm).

4.2.4 Observations

Weight of the sample = W = ----- g

Normality of the NaOH solution = N

Volume of NaOH (titre) = V = ----- ml

4.2.5 Calculations

1 ml of 0.05N NaOH = 1.6 mg of SO_2

$$\text{Therefore, V ml of N normal NaOH} = \frac{(V \times N \times 1.6)}{0.05} = (V \times N \times 32) \text{ --- mg } SO_2$$

Since (V x N x 32) mg SO_2 is present in W g of the sample

$$\text{Therefore, } SO_2 \text{ (mg) in 1 Kg of the sample (ppm)} = \frac{(V \times N \times 32)}{W} \times 1000$$

4.2.6 Results

SO_2 in the sample = ppm = mg per kilogram.

4.3 PRECAUTIONS

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.

Handle the all glass distillation apparatus very carefully. It may easily break because the joints are rigid.

EXPERIMENT 5 ESTIMATION OF BENZOIC ACID

Structure

5.1 Introduction
Objectives
5.2 Experiment
Principle
Requirements
Procedure
Observations
Calculations
Result
5.3 Precautions

5.1 INTRODUCTION

Benzoic acid is the second most common preservative added to fruit and vegetable products. Being insoluble in water, it is added as the soluble sodium benzoate. The method commonly followed for the estimation of benzoic acid involves conversion of the salt into benzoic acid by acidification, extraction into an organic solvent and titrating against alkali.

Objectives

After studying and performing this experiment, you should be able to

- determine the benzoic acid content of foods by the extraction and alkali titration method.

5.2 EXPERIMENT

5.2.1 Principle

In sodium chloride solution of the sample, the benzoic acid present is converted into water-soluble sodium benzoate by the addition of NaOH. When the sodium benzoate solution is acidified with excess HCl, it is converted into benzoic acid. The water insoluble benzoic acid is extracted with chloroform. The chloroform is removed by evaporation and the residue containing benzoic acid, which appears as leafy crystals, is dissolved in neutral alcohol and titrated against standard NaOH to phenolphthalein end point.

5.2.2 Requirements

Equipment and Apparatus

Separating funnel, 500 ml	–1
Beaker, 250 ml	–2
Volumetric flask, 250 ml & 500 ml	–1 each
Burette, 50 ml	–1

Pipette, 25 ml	–1
Measuring cylinders, 50 ml and 10 ml	–1 each
Water-bath	–1
Chemical balance	–1
Desiccator	–1

Whatman No. 1 or No. 4 filter paper circles

Chemicals and Reagents

Diethyl ether,

Hydrochloric acid – (1+ 3)

Anhydrous sodium sulphate

Ethyl alcohol

Phenolphthalein indicator – 0.1% in alcohol

Sodium hydroxide solution – 0.05 N

Ferric chloride solution – 0.5%

Sodium chloride – saturated solution

Sodium hydroxide – 10 % solution

Chloroform

Sodium chloride – powder

5.2.3 Procedure

a) Detection

Transfer homogenized sample into a separating funnel. Acidify with 1+3 HCl. Extract with solvent ether. Collect ether in a dry conical flask. Evaporate ether to near dryness and completely evaporate under a current of dry air. To the white leafy crystals of benzoic acid add few drops of 0.5% neutral $FeCl_3$ solution. A salmon coloured precipitate confirms presence of benzoic acid.

b) Determination

Take 50-75 g of the homogenized sample in a 500ml beaker. Add 200ml saturated solution of NaCl and 50g powder NaCl and stir well. Make the solution alkaline to litmus paper with 10% NaOH. Quantitatively transfer the solution to 250ml volumetric flask and make up to volume. Shake well. Let stand 2-3 hr. with frequent shaking. Filter through whatman No. 4 filter paper. Pipette 50ml filtrate into a separating funnel. Neutralize to litmus paper with HCl (1+3). Add 5ml in excess of the acid. Extract the solution thrice with 70, 50, 30 ml portions of chloroform. Shake funnel using rotary motion with each extraction. If emulsion forms, break it by (a) Stirring $CHCl_3$ layer with a glass rod, or (b) draw $CHCl_3$ layer into a second separating funnel and give one or two sharp shake. Pool clear $CHCl_3$ extracts and transfer to separating funnel. Wash the chloroform extract with

5ml water. Draw the $CHCl_3$ layer into a 250ml conical flask. (the $CHCl_3$ extract may be passed through a bed of cotton and anhydrous Na_2SO_4 to remove traces of moisture). Evaporate $CHCl_3$ to near dryness on water bath. Dry completely under a current of dry air. Keep the flask overnight in a H_2SO_4 desiccator. Remove and add 25ml neutral alcohol. Add 6-8 ml of water and one drop of phenolphthalein indicator and titrate against 0.05N NaOH.

5.2.4 Observations

Weight of the sample	= W = ---- g
Volume made up	= V = ---- ml
Volume taken for extracting with chloroform	= V_1 = ---- ml
Titre value	= V_2 = ---- ml
Normality of the NaOH	= N

5.2.5 Calculations

1000 ml 1N NaOH = 1 g equivalent of benzoic acid = 122 g benzoic acid

or 1 ml 1 N NaOH = 122 mg benzoic acid

Therefore ppm (mg per Kg) of benzoic acid in the product =

$$\frac{\text{Vol. of alkali} \times \text{Normality of NaOH} \times \text{Vol. made up}}{\text{Vol. Taken for } CHCl_3 \text{ extraction} \times \text{Weight of sample}} \times 122 \times 1000$$

$$= \frac{V_2 \times N \times V \times 122 \times 1000}{V_1 \times W} = \text{ppm}$$

5.2.6 Results

The result is expressed as benzoic acid in ppm.

5.3 PRECAUTIONS

The general precautions mentioned in the course ‘Introduction’ and those indicated in the experiments should be followed meticulously.

Never handle ether near a flame. The solvents should be handled only in a well-ventilated room or inside a hood with exhaust. Avoid inhaling the solvents directly.

EXPERIMENT 6 DETERMINATION OF HARDNESS OF WATER

Structure

6.1 Introduction
Objectives
6.2 Experiment: Hardness of Water by EDTA Method
Principle
Requirements
Procedure
Observations
Calculations
Result
6.3 Precautions

6.1 INTRODUCTION

Hardness of water is almost entirely due to the presence of calcium and magnesium salts. It is expressed as ppm (mg/ litre). Hardness values for water meant for different purposes have been specified.

The most common method for the determination of hardness of water is by the ethylenediamine tetra-acetic acid (EDTA) titration.

Objectives

After studying and performing this experiment, you should be able to

- determine the hardness of water.

6.2 EXPERIMENT: HARDNESS OF WATER BY EDTA METHOD

6.2.1 Principle

This method is based on the principle that when EDTA is added to a solution containing certain metal cations like calcium and magnesium, it complexes with them and make them unavailable for some reactions. One such reaction is the formation of wine-red colour between the cations and Chrome black T at a pH of 10. Therefore, for the determination of hardness of water, Chrome black T is added to the water at pH 10 ± 0.1 and titrated with EDTA solution till the disappearance of wine-red colour and formation of blue colour, which is the end point.

6.2.2 Requirements

Apparatus

Analytical balance

Hot air oven

Desiccator

Volumetric flasks, 500 ml and 1000 ml

Conical flask, 250 ml and 500 ml

White porcelain dish, 250 ml

Glass rods

Reagents

i) Borate buffer: Dissolve 20 g borax ($Na_2B_4O_7.10\ H_2O$) in about 400 ml distilled water. Dissolve 5 g sodium hydroxide and 2.5 g sodium sulphide (Na_2S) in 50 ml water, cool, mix with borax solution and dilute to 500 ml with distilled water.

ii) Indicator: Mix together 0.5 g of Eriochrome black T and 100 g sodium chloride and powder in a pestle and mortar.

iii) Methyl red indicator.

iv) 3N ammonium hydroxide solution.

v) Standard calcium solution: Dry calcium carbonate ($CaCO_3$) in an oven at 105^oC over night and cool in a desiccator. Weigh exactly 1 g into a 500 ml conical flask. Add dilute (1+1) HCl drop by drop until all the $CaCO_3$ has dissolved. Add 200 ml distilled water and boil for a few minutes to expel carbon dioxide. Cool, add a few drops of methyl red indicator and adjust to the intermediate orange colour by adding 3N ammonium hydroxide. Transfer to a 1 litre volumetric flask and make up to volume with distilled water (1 ml = 1 mg $CaCO_3$).

vi) Standard EDTA solution: Dissolve 4.0 g of disodium salt of EDTA in 800 ml of distilled water. Standardize against standard calcium solution. Adjust the dilution of the EDTA solution such that 1 ml = 1 mg $CaCO_3$. Store in a corning glass or plastic bottle to prevent extraction of salts from ordinary glass.

6.2.3 Procedure

Take 25 ml of the water sample in a porcelain dish and add 25 ml distilled water followed by 1-2 ml of the buffer. Add a small quantity of the Eriochrome indicator and stir with a glass rod to dissolve. If the water is hard, a red colour will be formed. Titrate slowly with the EDTA solution, stirring continuously until the red tinge disappears and a permanent blue colour is produced. If the water sample has low hardness, more volume of water may be taken for titration by adding proportionate volume of the buffer. The duration of titration should not exceed 5 min measured from the time of addition of buffer.

6.2.4 Observations

Volume of water sample taken for titration = V = ----- ml

Volume of EDTA solution required (titre) = V_1 = ----- ml

6.2.5 Calculations

Since 1 ml of the EDTA solution is equal to 1 mg $CaCO_3$, V_1 ml EDTA solution = V_1 mg $CaCO_3$.

As V_1 mg $CaCO_3$ is present in V ml of the water sample, the hardness of the water.

$$= \frac{V_1 \times 1000}{V} = \text{ppm } CaCO_3 \text{ or mg } CaCO_3 \text{ per litre.}$$

6.2.6 Results

Hardness of the water sample = ppm $CaCO_3$ or mg $CaCO_3$ per litre.

6.3 PRECAUTIONS

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.

EXPERIMENT 7 ESTIMATION OF RESIDUAL CHLORINE IN WATER

Structure

7.1 Introduction
Objectives
7.2 Experiment: Residual Chlorine by Iodometric Method
Principle
Requirements
Procedure
Observations
Calculations
Result
7.3 Precautions

7.1 INTRODUCTION

Water is routinely chlorinated to make it microbiologically safe. When chlorine is added to water other than distilled water, initially a small amount reacts with impurities in the water and does not show as residual chlorine. This is called the chlorine demand of the water, which has no germicidal effect. Chlorine added subsequently remains as the residual chlorine (free available and combined available chlorine), which is important for disinfection. The residual chlorine levels of water used for different purposes have been specified.

The most common method for routine estimation of residual chlorine is the iodometric method, which you will learn in this practical.

Objectives

After studying and performing this experiment, you should be able to

- determine the residual chlorine content in water; and
- follow the necessary sampling procedures.

7.2 EXPERIMENT: RESIDUAL CHLORINE BY IODOMETRIC METHOD

7.2.1 Principle

Chlorine liberates free iodine from potassium iodide solution in acidic pH quantitatively. The liberated iodine is determined by titration with standard sodium thiosulphate solution. As chlorine in aqueous solution is not stable, the determination of chlorine must be performed immediately after sampling. Care should be taken to avoid excessive exposure of the water sample to sunlight and agitation.

7.2.2 Requirements

Apparatus / glassware

Analytical balance

Burette, 25 ml

Volumetric flask, 100 and 250 ml

Conical flask, 250 ml

Measuring cylinder, 500 ml

White porcelain dish, 500 ml

Beaker, 250 ml

Reagents

i) Acetic acid, glacial.

ii) Potassium iodide crystals.

iii) Starch indicator.

iv) N sodium thiosulphate solution: Dissolve 24.8192 g of sodium thiosulphate ($Na_2S_2O_5$. 5 H_2O) in 200 ml water and transfer to a 1 litre volumetric flask and make up to volume. Standardize the solution with potassium dichromate. Weigh 0.20 to 0.23 g $K_2Cr_2O_7$ and transfer to a 250 ml beaker using about 150 ml water. Add 2 g potassium iodide and mix. Add 20 ml of 1 N HCl , swirl and allow to stand for 10 min. Titrate with the thisuphate solution adding 1 ml of 1% starch solution towards the end of titration and complete titration where the solution changes from blue green to light green.

$$\text{Normality of sodium thiosulphate solution} = \frac{\text{Wt of } K_2Cr_2O_7 \text{ (g)} \times 1000}{\text{Vol. of } Na_2S_2O_3 \text{ (ml)} \times 49.037}$$

Prepare 0.01 N working standard by diluting the 0.1 N thosuphate solution.

7.2.3 Procedure

Take a suitable volume of the water sample into a porcelain dish or beaker. For water containing 1 mg / litre or less chlorine take 1000 ml and for 1 to 10 mg / litre take 500 ml. The titre value of 0.01 N thiosulphate should not be more than 20 ml. Add 5 ml glacial acetic acid followed by 1 g potassium iodide, stir and titrate with 0.01 N thiosulphate solution until the yellow colour of the liberated iodine is almost disappears. Add 1 ml starch solution and titrate until the blue colour is discharged. Do not carry out the titration in direct sunlight. Blank titration can be carried out by taking equal volume of distilled water.

7.2.4 Observations

Volume of water taken for titration = V = ----- ml

Volume of thiosulphate solution required (titre) = V_1 = ----- ml

Normality of sodium thiosulphate solution = 0.01 N

7.2.5 Calculations

1000 ml 1N sodium thiosuphate = 35.46 g chlorine (i.e.1 g mole of chlorine)

or 1 ml 1N sodium thiosulphate = 35.46 mg chlorine

or V_1 ml of 0.1 N sodium thiosulphate = V_1 x 0.01 x 35.46 mg chlorine

Therefore, residual chlorine content of the water (mg per litre)

$$= \frac{V_1 \times 0.01 \times 35.46 \times 1000}{V}$$

$$= \frac{V_1 \times 354.6}{V}$$

7.2.6 Results

Residual chlorine content of the water sample = mg per litre or ppm.

7.3 PRECAUTIONS

The general precautions mentioned in the course ‘Introduction’ and those indicated in the experiments should be followed meticulously.

EXPERIMENT 8 DETERMINATION OF TOTAL SOLUBLE SOLIDS (°BRIX)

Structure

8.1 Introduction
Objectives
8.2 Experiment
Principle
Requirements
Procedure
Result
8.3 Precautions

8.1 INTRODUCTION

Total soluble solids may be determined by means of Refractometer. Brix is a measure of total soluble solids (TSS) in the case of pure sucrose solutions. Generally fruit juices contain more sugar than other soluble constituents, and hence, Brix provides useful guide of TSS or sugar content.

The concentration of sugar solutions can be determined conveniently for routine purposes using a refractometer. There are two types of refractometers viz. hand refractometers and Abbe's refractometers, the latter being tabletop instrument, which can measure both °Brix (TSS) of sugar solutions and also there is provision for maintaining constant temperature.

It should be noted that the refractometers are calibrated for sugar solutions and hence if the medium contains other soluble solutes in substantial quantities, there will be slight error.

Objectives

After studying and performing this experiment, you should be able to

- determine the °Brix of sugar solutions and TSS of food products.

8.2 EXPERIMENT

8.2.1 Principle

Refractometer measures total soluble solids (TSS) concentration based on the principle of refraction of light. When a ray of light travels obliquely from one medium to another, it is bent or refracted. The refraction occurs because light travels at slightly different velocities in different media, the extent being proportional to the density of the solution or the soluble solids concentration. The refractive index of a medium is defined as the ratio of the sine of the angle of incidence to the sine of the angle of refraction when a ray of monochromatic light is refracted from a vacuum (or, to a very close approximation, from air) into the medium. In a Brix refractometer, the refractive index is calibrated into °Brix readings. As refractive index is dependent on the density of the solution,

the measurements have to be made at a specific temperature (20°C) or suitable corrections have to be applied.

8.2.2 Requirements

Equipments

Hand Refractometer or Abbe's Refractometer

Thermostatically controlled water bath.

8.2.3 Procedure

Place few drops of the sample in between the prisms of hand refractometer and note the reading at the demarcation line. Apply temperature correction for readings taken at temperatures other than 20°C using the following table.

In the case of Abbe's refractometer, circulate 20°C water in the chamber enclosing the prism from a thermostatically controlled water bath. Place a few drops of the sample in between the prisms and allow the temperature to equilibrate and note the Brix reading, which gives per cent of sucrose sugar or TSS.

If sample is thick, squeeze it through cotton and place the drop in between the prisms.

8.2.4 Results

The readings are expressed as total soluble solids (TSS) = %.

Temperature corrections for readings of per cent sucrose in sugar solution by either Abbe or Immersion refractometer at temperatures other than 20°C.

Table 8.1: International temperature correction table, 1936

Temp (°C)	Percent Sucrose										
	0	5	10	15	20	25	30	40	50	60	70
Subtract from Per Cent Sucrose											
10	0.50	0.54	0.58	0.61	0.64	0.66	0.68	0.72	0.74	0.76	0.7
11	0.46	0.49	0.53	0.55	0.58	0.60	0.62	0.65	0.67	0.69	0.7
12	0.42	0.45	0.48	0.50	0.52	0.54	0.56	0.58	0.60	0.61	0.6
13	0.37	0.40	0.42	0.44	0.46	0.48	0.49	0.51	0.53	0.54	0.5
14	0.33	0.35	0.37	0.39	0.40	0.41	0.42	0.44	0.45	0.46	0.4
15	0.27	0.29	0.31	0.33	0.34	0.34	0.35	0.37	0.38	0.39	0.40
16	0.22	0.24	0.25	0.26	0.27	0.28	0.28	0.30	0.30	0.31	0.32
17	0.17	0.18	0.19	0.20	0.21	0.21	0.21	0.22	0.23	0.23	0.24
18	0.12	0.13	0.13	0.14	0.14	0.14	0.14	0.15	0.15	0.16	0.16
19	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08

Add to Per Cent Sucrose										
21	0.06	0.07	0.07	0.07	0.07	0.08	0.08	0.08	0.08	0.08	0.08
22	0.13	0.13	0.14	0.14	0.15	0.15	0.15	0.15	0.16	0.16	0.16
23	0.19	0.20	0.21	0.22	0.22	0.23	0.23	0.23	0.24	0.24	0.24
24	0.26	0.27	0.28	0.29	0.30	0.30	0.31	0.31	0.31	0.32	0.32
25	0.33	0.35	0.36	0.37	0.38	0.38	0.39	0.40	0.40	0.40	0.40
26	0.40	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.48	0.48	0.48
27	0.48	0.50	0.52	0.53	0.54	0.55	0.55	0.56	0.56	0.56	0.56
28	0.56	0.57	0.60	0.61	0.62	0.63	0.63	0.64	0.64	0.64	0.64
29	0.64	0.66	0.68	0.69	0.71	0.72	0.72	0.73	0.73	0.73	0.73
30	0.72	0.74	0.77	0.78	0.79	0.80	0.80	0.81	0.81	0.81	0.81

8.3 PRECAUTIONS

The general precautions mentioned in the course ‘Introduction’ and those indicated in the experiments should be followed meticulously.

EXPERIMENT 9 CONTAMINANTS: TIN CONTENT IN CANNED FOODS

Structure

9.1 Introduction
Objectives
9.2 Experiment: Tin Estimation by Iodimetry
Principle
Requirements
Procedure
Observations
Calculations
Result
9.3 Precautions

9.1 INTRODUCTION

Food contaminants are substances, which can cause harmful effects in the human system. They generally are pesticide residues, heavy metals, toxins and harmful microorganisms. Therefore, their detection and determination in foods is very important. However, the methods available for the determination of most of the contaminants require very sophisticated instruments and are time consuming. Therefore, most of these analyses are done in specialized laboratories and usually not done in the food processing industries.

One of the few estimations, which can be done easily, is tin content in canned foods. Canned food industry is one of the largest food processing industries. Dissolution of tin (can corrosion) into the canned product during storage is a common problem in canned foods. Excess tin dissolution causes discolouration and flavour change in the products and gives an unattractive appearance to can interior. Excess tin is also harmful to the human system. Therefore, maximum limits for tin content in canned foods have been specified.

Tin content in canned foods is usually determined by iodimetry.

Objectives

After studying and performing this experiment, you should be able to

- carry out ashing of food products for tin estimation; and
- perform the iodimetric estimation of tin.

9.2 EXPERIMENT: TIN ESTIMATION BY IODIMETRY

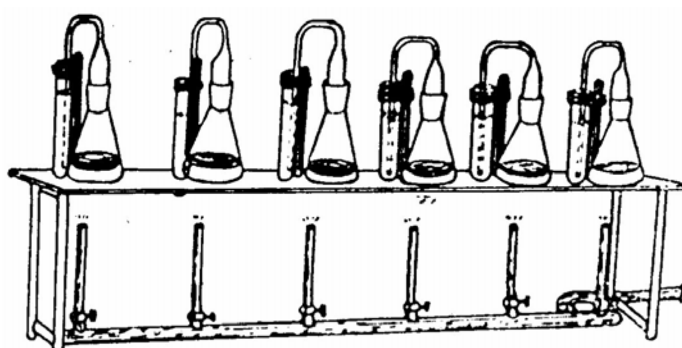
9.2.1 Principle

Canned food sample is acid digested (wet digested) to destroy the organic matter. Tin salts present in the digested sample are reduced with nascent hydrogen to stannous form. Titration of the stannous tin with potassium iodate quantitatively estimates tin.

9.2.2 Requirements

Instruments and Apparatus

Analytical balance	
Blender	
Kjeldahl digestion flask, 300 ml	–2
Pipettes, 10 ml	–2
Burette, 10 ml	–2
Volumetric flasks, 50, 100 ml, 500 ml, 1 lit	–1 each
Conical flask, 150ml with glass jointed (B24) capillary bent tube:	–2
Test tubes, 20 ml	–2
Glass beads	



Set of flasks used for reduction of tin salts

Reagents

i) Conc. HNO_3

ii) Conc. H_2SO_4

iii) Hydrogen peroxide

iv) 3N HCl: Dilute 294.6 ml conc.HCl to 1 litre with distilled water

v) Potassium iodide solution: Dissolve 0.2 g of KIO_3 and 3 g sodium bicarbonate in 100 ml of boiled and cooled water. Transfer to a reagent bottle. Add a few drops of HCl and shake. When effervescence ceases, insert stopper.

vi) Potassium iodate stock solution: Dissolve 5.3505 g KIO_3 in boiled and cooled water and make up the volume to 1 litre. Prepare working standard (0.005N) by diluting 5 ml to 100 ml.

vii) Antimont trichloride ($SbCl_3$) solution: Dissolve 1.5 g $SbCl_3$ in 50 ml HCl and dilute to 100 ml.

viii) Standard tin solution: Dissolve 0.5 g pure tin in 250 ml conc. HCl containing 2 drops of antimony trichloride solution and dilute to 500 ml (1 ml = 1 mg tin).

ix) Aluminium foil, pure.

x) 5% sodium bicarbonate solution.

xi) Starch indicator: 1% starch in 20% sodium chloride solution.

Note: The potassium iodide and iodate reagents should be freshly prepared.

9.2.3 Procedure

Empty the contents of the can immediately to a container after opening to avoid further tin pick up. Blend the product in a blender and digest a suitable quantity of the sample.

Wet digestion: Weigh 50 g sample and transfer to a Kjeldahl digestion flask using distilled water. Add a few glass beads and 10 ml conc. H_2SO_4 and 10 ml or more conc. HNO_3. Heat gently until the liquid darkens. Continue addition of HNO_3 in small proportions (1 to 2 ml) and heating until the solution fails to darken. At this stage, all the organic matter would have been oxidized. Cool and add 10 ml of hydrogen peroxide (30%) drop wise and heat until the digest is colourless. Cool and make up to 50 ml in a volumetric flask with distilled water.

Pipette out 20 ml of the digest into the 150 ml conical flask having glass joint. Add 1 drop of antimony trichloride solution, 30 ml of 3N HCl and about 0.3 g aluminium foil. Connect the flask by mean of B24 joint and a capillary bent tube to a "suck-back" test tube containing sodium bicarbonate solution. Sodium bicarbonate sucked into the conical flask, liberates CO_2 to maintain inert atmosphere.

Heat the flask gently until evolution of gas commences. When the aluminium foil has almost completely dissolved, heat again. Boil till the liquid is colourless. Cool the flask in ice water, disconnect the side tube and add about 4 ml potassium iodide solution along the sides of the flask from a pipette to wash down the digested solution. Add a few drops of starch indicator and titrate rapidly with 0.005 N potassium iodide solution to a blue end point. Run a blank determination with the regents at the same time. The blank titre should not be more than 0.2 ml of 0.005 N potassium iodate.

9.2.4 Observations

Weight of the sample taken: = W = ----- g

Volume of the digest made up to: = V = ----- ml

Volume of the digest taken for tin estimation: V_1 = ----- ml

Volume of potassium iodate required (titre): = V_2 = ----- ml

Blank titre: = V_3 = ----- ml

Normality of potassium iodate solution: = 0.005 N

9.2.5 Calculations

1000 ml 1 N KIO_3 = 59.35 g Sn (tin)

or 1 ml 1 N KIO_3 = 59.35 mg tin

Actual volume of the standard iodate required by the sample = (sample titre – blank titre) = $(V_2 - V_3)$

Therefore, tin content in the canned product (mg / Kg or ppm)

$$= \frac{\text{Actual titre} \times \text{Normality of } KIO_3 \times \text{Total volume of digest} \times 59.35 \times 1000}{\text{Volume of digest taken for tin estimation} \times \text{Weight of sample}}$$

$$= \frac{(V_2 - V_3) \times 0.005 \times V \times 59.35 \times 1000}{V_1 \times W} = \text{mg / Kg}$$

9.2.6 Results

Tin content in the product = ppm or mg per Kg.

9.3 PRECAUTIONS

The general precautions mentioned in the course ‘Introduction’ and those indicated in the experiments should be followed meticulously.

Handle the flask assembly with are to prevent breakage.