UNIT 1 AN OVERVIEW OF FOOD CHEMISTRY

Structure

1.0 OBJECTIVES

After reading this unit, you should be able to:

- explain what is food chemistry;
- state the historical developments in food chemistry; and
- discuss the different roles played by food chemistry.

1.1 INTRODUCTION

All of us need food for our survival and well being. We derive our food from the plant kingdom (like cereals, pulses, oilseeds, fruits, vegetables, root crops, etc.) and animal kingdom (like meat, fish, poultry, cow and buffalo, etc.). Do we know what are the constituents (nutrients) of the foods we eat? Do all foods contain the same constituents and in the same proportions or do they differ? Food chemistry has answers for all these. Today we have a fairly good knowledge of nutrient composition of all the common food materials and products so that we are able to plan a nutritionally balanced diet.

You have learnt the importance and methods of food preservation. Though we eat some foods in the raw form like fruits and vegetables, many others are stored for various length of time and consumed after cooking or converting them to some other forms as processed products like different types of wheat breads; various rice preparations, milk and meat products like cheese, yoghurt, sausages; fruit products like juices, jams, preserves; or dried and dehydrated products like mushroom powder. What changes take place in them after the food raw materials are harvested, processed and stored? Food chemistry deals with these.

Now we see numerous new ready to eat products on the grocer's shelf. They were not there 20 years ago. Steadily the numbers are increasing. How nutritious are these foods? We have become very familiar with the term 'food

adulteration' but how to know whether a particular food is adulterated and if so with what it is adulterated? Yes, a food analyst can find it out.

A general overview of these aspects is given in this unit. You will be learning more details of them in the subsequent units.

1.2 WHAT IS FOOD CHEMISTRY?

Food Science deals with the physical, chemical and biological properties of foods as they relate to stability, quality, processing, safety, nutritive value, wholesomeness, convenience and cost. Food Science is an inter-disciplinary subject involving primarily bacteriology, chemistry, biology and engineering. Food chemistry, a major aspect of food science deals with the composition and properties of food and chemical changes it undergoes during handling, processing and storage. Food Chemistry is intimately related to chemistry and biological sciences like biochemistry, botany, zoology and molecular biology. The primary interests of biological scientists include reproduction, growth and physiological and biochemical (morphological) changes that biological substances undergo under environmental conditions that are compatible with life. On the contrary, food chemists are concerned primarily with biological substances that are dead or dying (post harvest physiology of plants and post-mortem physiology of muscle) and changes they undergo when exposed to very wide range of environmental conditions. That is why a food chemist is concerned with conditions suitable for sustaining the residual life processes (post harvest physiology) for example fresh fruits and vegetables during their marketing.

Both in home scale food preparation and commercial food processing, food raw material are converted into convenient forms by pounding or milling of food grains, pulses, etc., oil extraction, extraction of fruit juices, etc. Food chemists are concerned with the chemical properties of these disrupted food tissues. In other words, food chemists have much in common with biological scientists, yet they also have interests that are distinctly different and are of utmost importance to human kinds.

1.3 HISTORY OF FOOD CHEMISTRY

The origin of food chemistry is as old as human civilization and shrouded in obscurity. Until the 20^{th} Century food chemistry did not have a clear identity and its early developments were associated with agricultural chemistry. During the period 1780-1850 many famous scientists made important discoveries, which laid the foundation of food chemistry.

Carl Wilhelm Scheele (1742-1786) is considered as one of the greatest chemist of all time who has done pioneering work in food chemistry. He isolated and studied the properties of lactose from milk, malic acid from apples and citric acid from lemon juice. He also tested a number of fruits for the presence of citric, malic and tartaric acids as well as various new chemical compounds. Antoine Laurent Lavoisier (1743-1794) also investigated the organic acid content a large number of fruits. He was perhaps the first to show that the process of fermentation could be expressed as a balanced equation. Theodore de Saussure (1767-1845) studied the CO_2 and O_2 exchange during plant

respiration and determined the mineral contents of plants by ashing. Joseph Louis Gay-Lussac (1778-1850) and Louis-Jacques Thenard (1777-1857) devised the first method to determine the percentages of carbon, hydrogen and nitrogen in vegetables. Sir Humphrey Davy (1778-1829) who isolated the elements K, Na, Ba, Sr, Ca and Mg wrote books on agricultural chemistry. In his book elements of Agriculture Chemistry (1813) he stated "the most essential vegetable substances consist of hydrogen, carbon and oxygen in different proportion, generally alone, but in some few cases combined with azote (nitrogen).

Jons Jacob Berzelius (1779-1848) determined the elemental composition of about 2000 compounds there by verifying the law of definite proportions.Justus von Liebig (1803-1873) classified foods as either nitrogenous (vegetable fibrin, albumin, casein, and animal flesh and blood) or nonnitrogenous (fats, carbohydrates, and alcoholic beverages). He is also credited for perfecting methods for the quantitative analysis of organic substances by combustion. His book "Researches on the Chemistry of Food" is considered by many as the first book on food chemistry.

By the first half of the twentieth century, most of the essential dietary substances, namely carbohydrates, proteins, lipids, vitamins, minerals etc. were discovered and characterised.

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 food chemistry?

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2. Name three scientists who have done pioneering work in the development of food chemistry.

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1.4 FUNCTIONS OF FOOD CHEMISTRY

Food chemistry, along with the other discipline of food science and nutrition play a vital role in ensuring nutritious and safe food to the human being. It is needless to state that to accomplish these roles, a thorough knowledge of the detailed chemical (nutritional) composition of foods is of prime importance.

1.4.1 Chemical Composition of Foods

As already mentioned, food chemistry has enabled us to know the nutrient composition of most of the common foods. This knowledge on their nutritional role and importance also accumulated. Nutrition studies showed that the human system require certain nutrients like carbohydrates, proteins and fats in large quantities and some others like vitamins and minerals in much smaller quantities. Therefore, the former group of nutrients were termed "major nutrients" and the latter 'minor nutrients'. Depending on the composition of foods, they were classified as 'carbohydrate rich (starchy) foods (e.g. Cereals, root crops), protein rich foods (e.g. meat, poultry and marine foods, legumes), fatty foods (oil seeds, fatty meat and fish) etc, Fruits and vegetables, in general are good sources of the minor nutrients viz. vitamins and minerals which have protective roles against certain deficiency diseases. Therefore, fruits and vegetables were classified as protective foods.

Knowledge on food composition and nutrition has also enabled planning and designing balanced foods suitable for different age groups, sex, convalescing, etc. Balanced food is a food formulation, which will provide all the nutrients in required quantities. Wherever, a food formulation is still deficient in certain nutrients, this knowledge enabled fortification to supplement them.

Today's nutrition literate consumers are demanding information on the nutrient content of the foods they consume. This has resulted in nutrition labelling of food products, which has become mandatory in some countries. Nutrition label provides information on the nutrient content of a particular food product and also what percentage of the Recommended Dietary Allowance of the nutrient is present in one normal serving of the product. The serving size is expressed in millilitres or grams. It is needless to state that nutrition labelling requires precise chemical analysis of the products.

In addition to the major and minor nutrients mentioned already, a number of bioactive compounds have been isolated from foods especially from fruits, vegetables and herbs. They are collectively termed 'Nutraceuticals' or 'Phytonutrients'. Some of them include: carotenoids, flavonoids, sulphides and thiols and phenolic cyclic compounds. Several of them have been shown to have antioxidative protection of the human body, suppression of cancer growth, improvement of vascular health, retardation of osteoporosis and control of cataracts. These developments have revived the old concept of 'Food as Medicine'.

The knowledge of the chemistry of food constituents has also enabled in modification of foods and food constituents. Production of fermented foods is an example of food modification. A large number of traditional fermented foods have been produced in different countries. Improvements in their processing steps and ensuring consistent quality have been possible due to the

knowledge in the chemical (biochemical) reactions. You will be learning more on this in subsequent units. Production of glucose syrup and high fructose syrup from starch, protein hydrolysates from proteins are examples of modification of food constituents.

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. Explain how the knowledge of food composition helps in formulating a balanced food.

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2. What is a balanced diet?

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3. Explain nutrition labelling.

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4. What is meant by nutraceuticals?

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1.4.2 Quality Changes in Foods

Quality of a food is a complex combination of several sensory and hidden (intrinsic) attributes. You are familiar with some of the sensory qualities like colour and appearance, firmness, mouth feel, flavour, taste etc. Alterations in these qualities of a food material, like for example, fruits results in value reduction and even rejection by the buyer. All these sensory quality changes are caused by chemical (or biochemical) reactions. For example, loss of the green colour of spinach on storage or cooking is due to degradation of chlorophyll. Similarly, browning of cut apples is initiated due to enzymatic oxidation of phenolic substances. Softening of fruits for example is due to the breakdown of pectins or toughening of meat is due to post mortem chemical changes resulting in pH reduction and tissue hardening. Flavour changes are also due to chemical reactions. For example, flavour change in fatty foods called rancidity is due to oxidation of unsaturated fatty acids. You will be learning more on these in subsequent units. The important point to be understood is that once you know the chemical cause of quality deterioration, it is possible to devise methods to control it.

The hidden quality factors of food are the nutrients content, and absence of adulterants and toxicants. Hidden quality cannot be perceived by sensory means. They have to be assessed by chemical means only. Among the hidden quality characteristics, nutritional quality changes are more important in storage and processing of foods. Among the nutrients, some of the vitamins are sensitive to processing conditions. For example, vitamin C (ascorbic acid) is very heat labile. Knowledge on the chemical kinetics of the reactions has enabled development of High Temperature Short Time processing technique and also non-thermal methods of processing. Breakdown of thiamine (vitamin B1) is well known. Therefore, sulphites are avoided for preservation of foods rich in thiamine. Nutritional evaluation of processed foods has been the subject of intense studies in recent times.

The quality changes in foods during processing and storage are due to two major factors namely product factors and environmental factors. Product factors include the chemical composition of a particular food, its pH, and available water content. Environmental factors of importance are temperature and time, light, access to microbial and insect attack and gas composition of the storage atmosphere. Altering the composition of food products to control quality changes is not easily possible except removal of water (drying) even though in a few cases it has been done. For example, to prevent browning of egg powder, glucose is removed from egg by enzymatically oxidising it.

Temperature effect on quality is to a great extent controlled by storage at low temperatures. One of the major functions of packaging is to prevent or reduce the effect of light on food quality. The effect of gas atmosphere on quality is equally important. When a food product is exposed to the atmosphere (containing about 79% N_2 and 21% O_2) several oxidative reactions take place. Examples are oxidation of fatty acids, oxidation of ascorbic acid, oxidative changes in flavour and browning reactions. In order to prevent the effect of oxygen in canned foods, the cans are exhausted (steam heating of cans filled with the product before sealing) to expel air, fruit juices are deaerated, antoxidants are added to fatty foods etc. You will be learning these techniques in other units. Another method is to alter the gas atmosphere, especially to

eliminate or reduce oxygen content in the gas atmosphere inside a package. You must have seen pillow packs (bloating pouches) of potato chips. They are filled with nitrogen to prevent browning and also to avoid physical damage to the chips. In the case of fresh fruits and vegetables, complete exclusion of oxygen is harmful. Knowledge of the biochemistry of plant respiration shows that reducing the oxygen concentration and increasing the carbon dioxide concentration can extend the storage life of these commodities. This has led to the development of Modified Atmosphere Packaging (MAP) and Controlled Atmosphere (CA) Storage of fresh fruits and vegetables. In MAP, mostly the gas composition is modified by the respiring commodities while in CA storage, the gas composition is modified physically by introducing or removing the respective gases.

Food adulterants and toxicants in foods have to be monitored to ensure food safety. This is a growing challenge to the food chemist.

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. Explain the chemical basis of two sensory quality changes.

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2. List the factors responsible for food quality changes.

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3. Explain how the effect of oxygen on quality change can be overcome.

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1.4.3 Safety Evaluation of Foods

Most scientific developments have both benefits and harmful effects. Food chemistry is no exception. The new knowledge gave ample opportunity to unscrupulous purveyors for food adulteration. Practically all foods are liable for adulteration. A few examples of food adulterants are given below.

Food products	Common adulterants
Milk	Cane sugar, starch, water
Spices and condiments	Sand, colouring matter, paddy husk, lead chromate, saw dust, argemone seed
Oils and fat	Mineral oil, argemone oil, tri-ortho-cresyl phosphate (TOCP), animal fat (tallow) in vegetable fat
Cereals and cereal products	Kesari dhal, colouring matter, talc, inorganic pigments
Beverages	Artificial sweeteners, unpermitted colouring matter, excessive preservatives
Coffee and tea	Cashew nut endocarp, date seed, tamarind seed/ powder, saw dust, added colour
Ice cream	Artificial sweeteners, unpermitted colours like metanil yellow
Synthetic vinegar	Mineral acids
Alcoholic drinks	Methanol, chloral hydrate

In addition to the intentional addition of harmful substance to the food (Adulteration), food contaminants coming into foods like agricultural chemicals (pesticide residues), heavy metals, etc. also need to be monitored.

As a consequence of this public health hazard, a new branch of food chemistry called analytical food chemistry developed essentially to detect adulteration and contaminants. Along side, new legislations to make adulteration unlawful also emerged which greatly expanded efforts by chemists to learn about the native properties of foods, the chemicals commonly used as adulterants and the means of detecting them. You will be learning more on these in subsequent units.

1.4.4 Waste Management

Food processing industries produce huge quantities of wastes – solid and liquid. The liquid wastes (effluents) are loaded with high concentration of sugars and other organic substances. They are quite often discharged into nearby streams. Environmentalists cry foul for justifiable reasons. The Environment Protection Act (EPA) has stipulated various parameters for safe discharge of effluents. This means that the effluent has to be treated suitably to degrade the constituents of the effluents to the safe limits. Therefore, knowledge of the chemical composition of the effluent is vital for designing the effluent treatment protocols.

About 10 to 50 per cent solid wastes are generated while processing food raw materials. They can be in the form of fruit and vegetable peel and pomace, rice husk and bran, milling wastes of other grains and legumes, slaughterhouse wastes and fish processing wastes. The easiest way to dispose them off is to dump them in the nearest land, compost or use them as fuel. A clear knowledge on the chemical composition of the wastes has enabled isolation of by products from them, some of them more valuable than the main product. For example, more than twenty by products are recovered from the peel and pomace of citrus fruits, which brings in more returns to the industry than the citrus juice concentrate, which is the main product. Some of the by products produced are pectin, peel oil, seed meal and oil, candied juice sacks etc. You will be learning more about by product utilization in another unit.

Microbial (also biochemical) conversion of the organic compounds in the wastes to biogas (mostly methane) is another possibility to utilise the food processing wastes. Biogas can be used as fuel.

1.4.5 Societal Roles

As the time progressed, food chemists had to assume greater responsibilities, the most important being involvement in social issues. As already mentioned the developments in food chemistry have created the monster called 'Food Adulteration'. It is the responsibility of the food chemist to contain it. The food chemistry knowledge has also opened up the possibility of using numerous chemicals called food additives to modify or improve the functional properties of foods. Some of them include: antioxidants, emulsifying, stabilizing and anticking agents, and colouring and flavouring agents. Many of the new food products will not have their functional properties without the addition of some of these additives. Their number is continuously increasing. There is considerable fear, often out of ignorance on their use. Food chemists can play the role of educating and advising the public on their usage. Today food chemists are playing a very complimentary role along with the physiologists, microbiologists, nutritionists, food scientists and technologists in providing safe foods to people in the form and place where they wish to have them.

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. List a few examples of food adulteration.

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2. Explain with two examples how fruit and vegetable processing wastes can be utilised profitably.

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3. Is there any societal role for a food chemist? If so what is the role?

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1.5 LET US SUM UP

Food chemistry plays a number of important functions in food and nutrition. Some of them include:

- Understanding the chemical nature of food constituents has enabled planning of food formulations suitable for different categories of people.
- Knowledge on the chemical reactions these compounds undergo has helped to control quality changes by developing suitable methods and techniques.
- Food chemistry knowledge assists in food modifications like food fermentations, modified products etc.
- Detects and identifies food adulterants there by ensure food safety.
- Enables proper and profitable management of food processing wastes.
- Advises on judicious use of food additives.

1.6 KEY WORDS

Food chemistry : Study of food constituents, their properties and changes during handling, processing and storage of foods.

Major nutrients : Carbohydrates, proteins and lipids.

Minor nutrients : Vitamins and minerals.

Nutrition labelling : Label of a packaged food product showing the content of its different nutrients per serving.

Nutraceuticals : Bioactive compounds like carotenoids, flavonoids, thiols present in some foods.

Hidden quality : Quality attributes like nutrient content, freedom from adulterants, toxicants that cannot be perceived by the human senses.

EPA : Environment protection act.

Enzymatic changes : Enzyme catalysed chemical reactions.

Biogas : Gas, mostly methane produced by anaerobic fermentation of organic wastes.

1.7 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   - Chemical composition, properties;
   - Chemical changes during storage, processing

2. Your answer should include the following points:

   - Scheele, Lavoisier, Liebig, Thenard

Check Your Progress Exercise 2

1. Your answer should include the following points:

   - Nutrient requirement of human system
   - Nutrient content of different foods
   - Mixing in suitable proportions

2. Your answer should include the following points:

   - Food formulation containing nutrients in required proportion.

3. Your answer should include the following points:

   - Nutrient content per serving

4. Your answer should include the following points:

   - Bioactive compounds
   - Carotenoids
   - Flavonoids
   - Thiols

Check Your Progress Exercise 3

1. Your answer should include the following points:

   - Chlorophyll degradation in spinach and green colour change
   - Pectin degradation and fruit softening

2. Your answer should include the following points:

   - Product factors
   - Environmental factors

3. Your answer should include the following points:

   - Exhausting
   - Nitrogen packing
   - Modified Atmosphere Packaging
   - Controlled Atmosphere storage

Check Your Progress Exercise 4

1. Your answer should include the following points:

   - Starch, sugar, water in milk
   - Paddy husk, saw dust in spices
   - Dates seed, tamarind seed in coffee and tea
   - Mineral acid in vinegar

2. Your answer should include the following points:

   - Pectin, peel oil
   - Biogas

3. Your answer should include the following points:

   - Educating and advising the public on the safe use of food additives.

1.8 SOME USEFUL BOOKS

1. Braverman, J.B.S. (1963) Introduction to the Biochemistry of foods, Elsevier Publishing Company, Amsterdam, London, New York.

2. Meyer L.H. (1969) Food Chemistry, Van Nostrand Reinhold Company, New York, Cincinnati, Toronto, London, Melbourne.

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

UNIT 2 AN OVERVIEW OF FOOD PHYSIOLOGY

Structure

2.0 OBJECTIVES

After going through this unit, you should be able to:

- know the different stages of growth in the life of fruit;
- understand the physiological changes in fruits and vegetables that take place after harvest;
- tell how senescence can be delayed; and
- mention the structural and compositional changes that take place after harvest.

2.1 INTRODUCTION

Fruits and vegetables are living tissues and remain alive even after harvest. They undergo considerable morphological and bio-chemical changes during growth and following harvest. The visual changes in colour are most prominent indicator of ripening. Many of the quality parameters of harvested produce are affected by pre-harvest factors starting from planting, planting density, irrigation and hormonal treatment. To obtain a produce with good quality all these factors must be controlled.

2.2 MORPHOLOGICAL CHARACTERISTICS

The life of fruit and vegetable crops can be divided into three major physiological stages following germination- growth, maturation and senescence. Growth involves cell division and subsequent cell enlargement, which accounts for final size of the commodity. Maturation usually commences before growth ceases. Growth and maturation are often collectively referred to as development phase. Senescence is defined as the period when anabolic (synthetic) bio-chemical processes give way to catabolic (degradative) processes, leading to ageing and finally death of the tissue. Ripening is considered to begin during the later stages of maturation and to be the first stage of senescence. Development and maturation of fruit are

completed only when it is attached to the plant, but ripening and senescence may proceed on or off the plant. But in many plants, a growth after harvest has been observed which is considered undesirable. Elongation and toughening in asparagus, toughening in beans, sprouting in potato and onions are the examples of such growth that occurs after harvest.

Growth and Development

Many tiny individual cells make up the plant body. Each cell consists of living system [protoplasm] and usually a cell wall. The protoplasm is the most significant part of the plant. It is in fact a factory, which manufactures the products of the plant, including the walls of the cell themselves. It is composed of water, salts, sugars, proteins, fats, enzymes, vitamins, growth regulators and a complex of other materials. All of this is organised, through a special portion of the protoplasm [nucleus], into a living unit. As the plants grow and develop, individual cells divide and differentiate into particular kinds of cells and groups of cells, called tissues and organs performing special functions. This addition of cells and the increase in biomass is called growth. An essential organ of a plant is fruit, which develops from another organ of the plant called flower.

Development of fruit and seed

The flower is a group of specialized leaves concerned with the development of structures, which lead to sexual production. A flower consists of four parts:

i) an outer whorl of sepals, usually green, collectively known as the calyx;
ii) petals, usually coloured, which together are called the corolla;
iii) stamens, which produce the pollen grains and male germ cells; and
iv) the pistil, which consists of one or more sections [carpels] bearing the female germ cell and later the seed.

The flower parts are mounted on a portion of the stem known as receptacle. During the formation and development of flowers of any part of the plant, growth regulators play an important role.

A fruit develops from the ovary of pistil. It may consist of a single carpel, as in cherry or of several carpels, as in tomato. Also other parts of the plant may be associated with the fruit, as the enlarged receptacle of the strawberry, the sepals of mulberry and the stem of the pineapple. Attached to the inner edges of the carpel and enclosed within them are the parts (ovules), which develop into seeds.

Initiation of fruit development

Male cells [gametes] from the pollen grains are transferred to the tip (stigma) of the pistil and grow down (through the style) to fertilize the female cells, which are formed in the ovules enclosed in the ovary. The process by which the pollen grains are transferred to the stigma of pistil is called pollination and the process of the fusion of male and female cells (gametes) is called fertilization. The fertilized cell called zygote further grows, divides, differentiates and develops into a new plant (embryo) enclosed in the seed coats. Later, the ovary develops into a fruit. Therefore, the fruit is a matured or

ripened ovary. All these processes are carried through the influence of certain growth regulatory compounds called plant-hormones.

Physiology of growth and development

Carbohydrates, which are essential to both plant and animal life are produced in the leaves in a process known as photosynthesis, in which the green colour pigment of the plant (chlorophyll) utilizes light energy to form simple carbohydrate materials (as sugars) from water and carbon dioxide of the atmosphere.

$$6\ CO_2 + 6\ H_2O + 673{,}000\ Cal \leftrightarrow C_6H_{12}O_6 + 6\ O_2$$

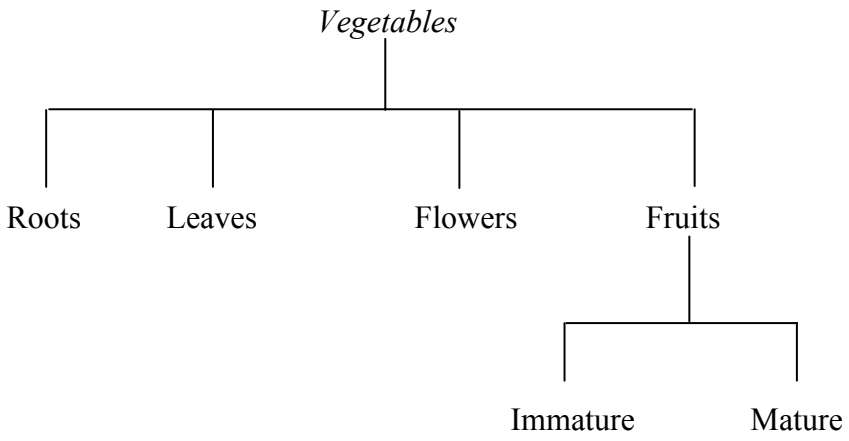
Carbohydrates are moved from the leaves to other parts of the plant. The most important tissues involved in translocation are the xylem and phloem. It is believed that the carbohydrates travel through phloem, while water and minerals travel mainly through xylem.

Food may be stored in various storage organs, such as roots, tubers, rhyzomes, bulbs, corms, fruits and seeds. Storage may occur at different seasons of the year and may in some plants be controlled by the length of the day, the length of the night period and the day & night temperatures. In many plants that live for more than one season (perennials), accumulation in the underground storage organs takes place at a rapid rate in the fall of the year.

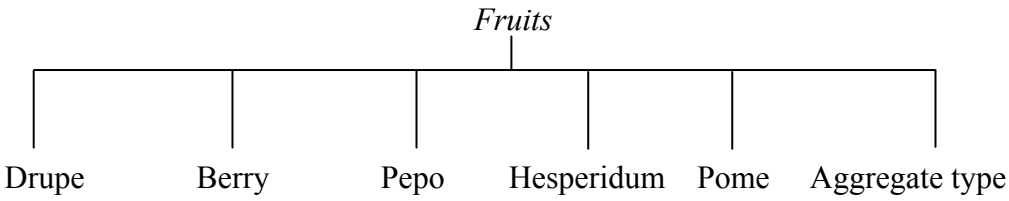
Fruits and Vegetables

Fresh fruits, as well as fresh vegetables are essential components of human diet. Both contain a number of nutritionally important compounds, such as vitamins, which cannot be synthesized by the human body; vitamin C is the most important essential nutritive substance found mainly in fruits and vegetables.

The fruits are used as a table commodity whereas the vegetables are usually cooked and then used as food. Some of the vegetables are "fruit-vegetables" and most of the vegetables are the other vegetative organs of the plant that include root, stem, flower, shoot, leaves and associated parts. On the basis of the parts of plants used as food, the vegetables are classified in the following groups:



Similarly, depending on the parts of the ovary wall [pericarp-epicarp, mesocarp and endocarp] developing into flesy and succulent organs of the fruits are classified as under:



2.3 POST-HARVEST PHYSIOLOGY OF FRUITS AND VEGETABLES

Fruits and vegetables are highly perishable commodities with active metabolism during the post-harvest period. Proper handling plays an important role in increasing food availability. On removal from the parent plant, vegetative parts, such as fruits, roots, stems etc are deprived of their normal supply of minerals, water, and also in some instances, simple organic molecules [e.g. sugars, hormones] that normally would be translocated from other parts of the plant. Innumerable physiological and bio-chemical processes are initiated and continued in the edible plant tissues at the time of harvest. Although the photosynthetic activity is negligible, most tissues remain capable of transforming many of the constituents already present in them. The diversity of metabolic shifts, which are specific to a given commodity [and often variety] are manifest in events such as ‘rotting’, ‘ripening’, ‘sprouting’, ‘scald’, ‘brown core’, ‘hard core’, ‘toughening’, and ‘yellowing’.

The kind and intensity of physiological activity in detached plants determines their storage longevity. Some plant parts, such as seeds, fleshy roots, tubers, bulbs are morphologically and physiologically adapted to maintain the tissue in a dormant state until environmental conditions are favourable for germination or growth. Metabolic activity, though depressed, is not completely halted in such tissues. Fleshy fruits are unusual in that maturation is followed by a ripening process, which is associated with the development of optimal eating quality.

The diversified visible physiological changes, like sprouting, browning, toughening etc are desirable in some commodities and undesirable in others in relation to the eating quality. Almost all such changes are observed during a most important physiological process called ripening.

Physiology of Ripening

The term “ripening” is generally referred to the physical and bio-chemical changes taking place in the fruits after the cessation of growth till the onset of senescence and decay. The ripening process is dependent upon maturity, since a given stage of development must be attained before ripening proceeds. The process of ripening continues while the fruit is on the tree, but the damage caused by the birds, insects etc makes it uneconomical to allow the fruits to ripen on the tree. Hence, the fruits are usually harvested at the horticulturally mature stage. Fruits being living entities continue to carry on the normal physiological processes resulting in the ripening and finally decay or death of

the fruit even after they are separated from the parent plant/tree. Most of the fruits show the ripening changes after harvest with a few exceptions like grapes, which are to be ripened only on the vine, as they do not ripen well after harvest. Since the changes taking place in a fruit during ripening greatly influence the eating quality and the monetary value of the commodity depends on it, a detailed knowledge of the physiology and biochemistry of ripening is desirable for the successful storage and marketability of fruits.

Changes during Ripening of Fruits

Important changes occurring in the fruits during ripening include – 1) Respiration, 2) Transpiration, 3) Carbohydrates, 4) Texture, 5) Flavour, 6) Pigments, 7) Organic acids, 8) Nitrogenous compounds, 9) Tannins, and 10) Enzyme activity.

1. Respiration

Fruits and vegetables of different species differ as to the nature and the rate of the changes taking place but most of them share a respiratory pattern known as "the climacteric". In some fruits, it has been observed that the respiration rate increases with the ripening to a maximum level called as 'Climacteric' peak which is followed by a steady decline in respiration rate, often called senescence. The fruit attains the eating ripe stage at the climacteric peak or sometime after the peak, depending on the species and to some extent temperature and composition of the atmosphere in which the commodity is stored. All other fruits showing no such respiratory pattern are called non-climacteric. In citrus fruits [oranges and lemons], the maturation and ripening progress slowly and the respiratory activity tends to decline following harvesting of commercially mature fruits.

Another important criterion for distinguishing a 'Climacteric fruit' from a 'non-climacteric fruit' is the response to ethylene application. It is well known that ethylene has an enhancing effect on fruit respiration. Biole (1954) showed that a non-climacteric fruit would react to ethylene treatment at any stage of its preharvest or postharvest life, whereas a climacteric fruit will exhibit a respiratory response only if ethylene is available during the pre-climacteric stage, and becomes insensitive to ethylene treatment after the onset of the climacteric rise.

There is a fairly consistent relationship between storage type of fleshy plant tissues and respiration, e.g. peas with a high respiration rate [50 mg CO_2/kg/hr] have a short storage life [1 week at 5°C], while turnips with low respiration rate [6 mg CO_2/kg/hr] have a long storage life [16-20 weeks at 5°C]. The shelf life of a given commodity can be greatly extended by placing it in an environment, which retards the rate of respiration. In other words, the environment modification by refrigeration and carbon dioxide/oxygen (CO_2/O_2) conc. controlled atmosphere will provide a direct effect on the determination of shelf life of the commodities.

Respiratory quotient

From measurements of CO_2 and O_2, it is possible to evaluate the nature of respiratory process. The ratio of CO_2 to O_2 is termed the respiratory

quotient [RQ]. This is useful in deducing the nature of the substrate used in respiration, completeness of the respiratory reaction and degree of anaerobic or aerobic process.

2. *Transpiration*

When the fruit is picked and severed from the plant, water no longer flows into the fruit, although the loss of water continues. This process of loss of water in vapour form is known as transpiration. By the time the fruit loses 5% of its original weight, it appears shriveled enough to lose its eye appeal as well as the eating quality. The fruit becomes unattractive because of wrinkled appearance. Hence, this loss of water due to transpiration should be checked in order to maintain its marketability. Judicious application of wax emulsion or other skin coating or pre-packaging with thin paper or cling films could successfully reduce such water losses. On the other hand, in some fruits during ripening, the water content of the pulp increases and the peel decreases, as in the case of banana, which makes the fruit better in eating quality.

2.4 STRUCTURAL CHANGES DURING GROWTH AND RIPENING

A number of changes occur in fruits during ripening. Softening of tissue during ripening is attributed to the changes in cell wall thickness, permeability of plasmalemma, and amount of intercellular spaces. The change in colour of ripened fruit is due to transformation of chloroplast to chromoplast. Although the structure of mitochondria is maintained during growth and ripening, it may degrade in overripe stage. Cuticle deposition increases continuously during ripening but the epidermal hairs either reduce in number or disappear completely.

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 the different phases of growth in the life of a fruit?

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2. How does softening of tissues occur in fruits during ripening?

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3. Why colour of a commodity changes during ripening?

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4. What is senescence?

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2.5 COMPOSITIONAL CHANGES DURING GROWTH AND RIPENING

Carbohydrate

Sugars are important for pleasing fruit flavour (sugar acid ratio), attractive colour and texture. As the ripening starts these sugars undergo metabolic transformation both quantitatively and qualitatively. Most of the soluble carbohydrates are metabolized completely as the fruit ripens. Pectic substances and cellulose are the reserve carbohydrates that also serve as potential sources of acids, sugars and other respiratory substances during ripening.

In the process of ripening many changes occur in the carbohydrates fraction of fruit during the climacteric and senescence. Green or raw fruit usually contains starch in abundance, but is short in soluble sugars that provides sweetness to it. During ripening, the starch is enzymatically [hydrolysis by alpha- and beta-amylases] converted into sugars. Thus, the major bulk of carbohydrate fraction of a fully ripened fruit consists of sugars. The sugars commonly found in fruits are glucose and fructose [invert sugars] and sucrose.

Organic Acids

The organic acids are among the major cellular constituent undergoing changes during ripening. In most of the fruits there is a considerable decrease in the acidity of fruits during ripening.

The sourness of fruits is due to the presence of organic acids like citric, malic, succinic, tartaric, oxalic etc. These acids usually decide the quality of fruits as the blending of sugar and acids render the fruits tasty, besides flavour. Though these organic acids are present in varying amounts in raw or unripe fruits, but the concentration considerably changes as the fruits ripen. In fruits like oranges, the acids are converted enzymatically into sugars rendering them sweet as they ripen, whereas there is no change in lemons. So they remain sour till they start decaying. But, in some fruits like mangoes, there is a considerable decrease in acidity when the fruits fully ripen. This is probably due to the utilization of these acids in respiration through Kreb's Cycle. Generally, in fruits the total acidity shows a decrease with the increase in ripeness of the fruits.

Amino Acids and Proteins

A major turnover of amino acids in mango takes place during ripening, whereas in carombola it continuously declines. Small increases in protein content were also observed in mango, tomato and avocado.

The nitrogen content of fruit is due to proteins forming insoluble fraction and the soluble fraction comprised of amino acids. The total nitrogen content of fruits at the early stages is high, but with the advancement in growth, shows gradual decrease. This is probably due to the increase in other constituents like water, starch, sugar, organic acids etc. During ripening, the total nitrogen may show a further decrease in some cases.

Lipids

Phospholipids occur in the cytoplasm and in many structural units of plant tissues. They are physiologically more important than neutral lipids in storage organs. Considerable increases in the level of total lipids and fatty acids have been observed in ripening mango in contrast to many fruits and vegetables. However in fatty fruits of avocado the oil composition during maturation remains more or less constant.

Chlorophyll

Disappearance of green colour marks the initiation of ripening in most of the fruits. Chlorophyll content of ripening fruit decreases universally.

Carotenoids

A dramatic synthesis of carotenoids occur during the last step of ripening. It has been reported that the levels of carotenes, free geraniol, mevalonic acid, all precursors of carotene biosynthesis increases progressively during ripening.

Other Pigments

The colour imparted to raw or ripe fruits and vegetables are due to presence of various pigments. The pigments of different tissues are the chlorophyls (green), anthocyanin [reddish to purple], flavonoids [yellow], leucoanthocyanins [colorless], tannins [colorless to yellow or brown], betalains [red], quinones and xanthones [yellowish] and carotenoids [yellow and red].

During storage some of these pigments undergo considerable changes. Carotenoids formation and destruction may be affected by the storage conditions. In certain instances, these reactions are stimulated by O_2, inhibited by light and high temperature. Carotenoids include lycopone, Beta, Gamma carotenes and are synthesized enzymatically in the fruits. Anthocyanin synthesis is stimulated by light and is often affected by temperature. Purple colour of red cabbage intensifies when stored below 10^{o} C. Chlorophyll degradation is accompanied by synthesis of other pigments as the fruits ripen. Chlorophyll metabolism is markedly influenced by environmental parameters, such as light, temperature and humidity and the effects of these factors are specific for the tissues. For example, light accelerates degradation of chlorophyll in ripening tomatoes and promotes formation of the chlorophyll pigment in cold stored potatoes.

Tannins

The tannins and other polyphenolic constituents are present in abundant quantities in immature, raw or developing fruits. As the maturity and ripening progresses the total polyphenolic content reduces gradually.

Pectic Substances

The most obvious changes during ripening of fruit are the alteration in texture. The plant cell wall is made up of cellulose fibrils embedded in a matrix consisting largely of pectic substances, hemicellulose, proteins, lignins etc and water. Cell wall and middle lamella components increase during development of fruits, but as the fruit ripens the content of soluble pectates and pectinates increase, while total pectic substances decrease.

The cell walls are surrounded by parenchymatous cells, which will absorb water and generate hydrostatic pressure within the living cells. This is called turgor pressure that gives the desirable property of crispness to the commodity. During storage, the loss of moisture due to transpiration and respiration results in the loss of crispness or the turgidity of the commodity. In addition, the changes in the pectic substances – [which form a component of the cell walls of the fruit] – account for the firmness of the fruits. During ripening, the protopectin, which is insoluble and forms, the middle lamella of the cell wall, decreases in quantity and the soluble pectin content rises, thereby making the flesh less firm or soft. A decrease in the chain length and loss of methyl groups

of proto-pectin probably occurs during ripening, accounting for the rise in soluble pectin. This is brought about enzymatically mainly by the enzymes pectinase and pectin methyle sterase.

Volatile Products

Each fruit has specific aroma which ripened fruit emanates. Although different fruits vary in nature of volatile compounds, they are emitted in noticeable amount only when the fruit starts ripening. Although the degree of maturity is the main physiological factor affecting aroma production, the aroma composition is also affected by environmental conditions during maturation. In overripe fruits mostly alcohol and esters are formed when fermentation develops.

One of the marked differences between an unripe and ripe fruit is the intensity of flavour of the fruit. The flavour of fruits or vegetables are considered to originate by the presence of basic constituents, such as carbohydrates (particularly mono- and disaccharides], proteins [particularly free amino acids] and fats [triglycerides or their derivatives], as well as vitamins and minerals. These constituents are produced through photosynthetic and related metabolic activities occurring in the commodities. Some volatile compounds may exist in the tissues as such but in some it may be formed enzymatically upon rupture of cells or by microorganisms. Besides ethylene, a number of other volatile odorous constituents like amyl esters of formic, acetic, valeric and caprylic acids have also been identified. These organic emanations produced during ripening of fruits contribute to the aroma of fruits and hence are of considerable importance from the standpoint of fruit quality.

Enzymes

Enzyme action is responsible for many chemical and physical effects during ripening. Softening of fruits, conversion of starch to sugar or vice versa, changes in amino acid content, and enzymes bring changes in color.

Most of the bio-chemical changes occurring in fruits during ripening can be attributed to enzyme reactions. The change from starch to sugar, sucrose to invert sugar or protopectin to pectinic acid are all due to enzymic reactions.

Oxidative enzymes like catalase and peroxidase were shown to have increased to a considerable extent in ‘Alphonso’ and ‘Neelam’ varieties of mangoes during ripening as indicated by the higher rate of respiration. Similarly, glycolytic and hydrolytic enzyme activity were also found to increase in ripening mangoes, particularly during climacteric and post-climacteric stages. Transminase activity also increased in mangoes, resulting in the increased amounts of amino acids. Chlorophyllase activity followed the climacteric pattern in bananas, but suggested that the ensuing chlorophyll degradation may not be relevant to ripening. Other enzyme that increases in activity during ripening and following respiratory climacteric is fatty acid synthatase in Avocado fruit.

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 the effect of planting density on post harvest quality of the horticultural produce?

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2. What is the effect of pre-harvest fertilization on post harvest quality of horticultural produce?

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3. How pre-harvest diseases affect the quality of fresh produce?

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4. What are the effects of low water on fruit quality?

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2.6 LET US SUM UP

The quality of fresh produce depends on many factors during growth and after harvest. The cultural practices followed before harvest has marked effect on fruit quality. A number of physiological and bio-chemical changes take place during growth of the produce that continues after harvest also. Therefore it is important to follow good pre and post harvest practices to extend the shelf life and maintain the quality of horticulture produce.

2.7 KEY WORDS

Morphological changes : Visible changes on the outer surface of the product.

Chemical changes : Changes in composition of the product.

Ripening : The advance stage in the development at which fruit and vegetable are suitable for consumption/ utilization.

Growth : Gradual development towards maturity (increase in size, weight, etc.).

Maturation : Becoming full grown or fully developed.

Senescence : Beginning of final phase in the life of plant.

Cultural practices : A set of operations used for raising a crop in the field.

Planting density : Number of plants per unit area.

Pruning : To cut away or remove unnecessary plant parts.

Thinning : Reduction of population of plants.

2.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answers should include following points:

   - Growth
   - Maturation
   - Senescence

2. Your answers should include following points:

   - Cell wall thickness
   - Plasmalemma
   - Intercellular spaces

3. Your answers should include following points:

    - Chloroplast
    - chromoplast

4. Your answers should include following points:

    - Anabolic
    - Catabolic
    - Aging
    - Death

Check Your Progress Exercise 2

1. Your answers should include following points:

    - Light availability
    - Fruit size

2. Your answers should include following points:

    - Mineral deficiency
    - Mineral toxicity

3. Your answers should include following points:

    - Reduced yield
    - Poor quality

4. Your answers should include following points:

    - Fruit size
    - Splitting
    - Disorders

2.9 SOME USEFUL BOOKS

1. Kader, A.A. (1992) Post-harvest Technology of Horticultural Crops. University of California Publication No 3311, Oakland, Calf.

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3. Ryall, A.L. and Lipton, W.J. Handling (1979) Transportation and Storage of fruits and vegetables. Vol. 1, Fruits and Nuts, AVI Pub. Co.

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5. Salunkhe D.K., Kadam, S.S. (1995) Handbook of fruit science and technology: Production, composition, storage, and processing. Marcel Dekker, Inc. 270 Madison Avenue, New York, New York.

6. Salunkhe, D.K. and Desai, B.B., Boca Raton, N.W. (1984) Post-harvest biotechnology of vegetables. Vol. I and II CRC Press, Inc., Florida,

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8. Weichman, J. and Basel (1987) Post-harvest physiology of vegetable. Marcel Dekker Inc., New York.

9. Wills, R.B.H.; Lee, T. H.; Graham, D.; McGlasson, W.B. and Hall, E.G. (1981) Post-harvest: An introduction to the physiology and handling of fruits and vegetables. AVI Publishing Co. Westport, Conn.

UNIT 3 FOOD CONSTITUENTS – CARBOHYDRATES AND LIPIDS

Structure

3.0 OBJECTIVES

The aim of this unit is to introduce you to the chemistry of two major constituents of foods viz. carbohydrates and lipids. After reading this unit you will become familiar with the following aspects:

- *carbohydrates:* Monosaccharides, disaccharides, oligosaccharides and polysaccharides; their occurrence in foods, structure, properties and uses in food industry; and
- *lipids:* Occurrence, chemical properties, fatty acids and their properties and their changes during processing and storage of foods.

3.1 INTRODUCTION

Carbohydrates along with lipids are the primary source of energy for the human system. Carbohydrates are widely distributed in plants and to a limited extent in animals. They under go various changes during processing of foods. Lipids are present both in plant and animal foods. They also undergo physical and chemical changes during processing and storage of foods. Therefore, an understanding of the chemical nature of these substances is very important for preserving foods. In this unit you will be learning the chemical aspects of carbohydrates and lipids.

3.2 CARBOHYDRATES

The first products of photosynthesis in plants are carbohydrates. These compounds, in one form or another, constitute more than one half of the organic matter on earth. The major part of plants is built of carbohydrates while the animal world contains rather limited amounts of them.

3.2.1 Occurrence

Carbohydrates occur in plant and animal tissues as well as in microorganisms in different forms and quantities. They along with oils and fats are the primary source of energy for the human system. In plants, a wide variety of monosaccharides and oligosaccharides occur. Starch is the main storage carbohydrate in root crops and cereals. Similarly, the 'building bricks' or structural carbohydrates of plants are cellulose, hemicellulose and pectin. Some plants, as well as seaweeds and microorganisms produce different types of gums, which are a different group of polysaccharides. Fruits contain predominantly the monosaccharides, glucose and fructose, and the disaccharide sucrose as well as other mono and oligosaccharides in smaller concentrations. Animal foods have mainly glucose and the storage carbohydrate is glycogen. In milk it is almost exclusively the disaccharide lactose. Another group of polysaccharides called dietary fibre (pectin, hemicelluloses, pentosans, etc.) are gaining considerable health importance. Table 3.1 gives an idea of different types of carbohydrates present in different types of foods.

Table 3.1: Average carbohydrate composition and Water content of some foods

Carbohydrate	Cereals		Legumes		Fruits		Vegetables	
	Wheat (%)	Rice (%)	Beans (%)	G.nut (%)	Apple (%)	Orange (%)	Potato (%)	Tomato (%)
Monosaccharides								
D-Fructose	0.1	-	-	-	5.0	1.5	0.1	1.6
D-Glucose	0.1	-	-	-	2.0	2.5	0.1	1.2
Oligosaccharides								
Sucrose	1.0	-	3.0	4-5	3.0	4.6	-	1.0
Polysaccharides								
Starch	71.0	79.0	10.0	15.0	-	-	20.0	-
Pectin	-	-	-	-	0.6	1.3	-	0.3
Water	14.0	13.0	11.0	2.0	84.0	86.0	79.0	93.0

3.2.2 Nomenclature

Carbohydrates were first named according to their natural sources eg. cane sugar, beet sugar, malt sugar, grape sugar etc. Subsequently they got trivial names often from a prefix related to the source followed by the suffix '-ose'. Examples are: fructose (fruit sugar), maltose (malt sugar), lactose (milk sugar), xylose (wood sugar) etc. Though these names do not provide any information on their chemical structure they are still being used widely.

3.2.3 Classification

Carbohydrates are classified into monosaccharides, oligosaccharides and polysaccharides.

Monosaccharides

The simple carbohydrates, the monosaccharides, are neutral, crystallisable substances, which are readily soluble in water. Most of them are sweet covering a wide range of sweetness. Monosaccharides may be divided

chemically into polyhydroxy aldehydes (aldoses) and poly hydroxy ketones (ketoses). Depending on the number of constituent formaldehydes (CH2O), these sugars are classified as:

Bioses (CH2O)2;	C2H4O2 (example: glycol aldehyde)
Trioses(CH2O)3;	C3H6O3 (example: glyceraldehyde)
Tetroses (CH2O)4;	C4H8O4 (examples: Erythrose, threose)
Pentoses (CH2O)5;	C5H10O5 (examples: Ribose, rafinose,)
Hexoses (CH2O)6;	C6H12O6 (examples: glucose, mannose, gulose, galactose, fructose, sorbose)

The monosaccharides, which are of primary importance in foods, are hexoses and pentoses.

Hexoses: Five hexoses, three of them aldoses (glucose, mannose and galactose) and two ketoses (fructose and sorbose) are found in the free state in plants. The simple open chain formula of these hexoses (propounded by Fisher) are shown in Figure 3.1.

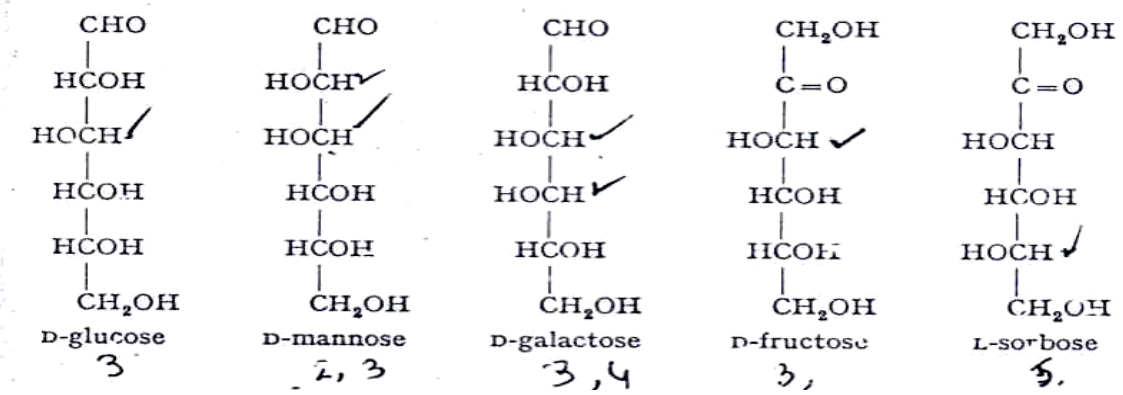

Figure 3.1: Open chain formulae of some hexoses

You will notice that the differences in the structures of these hexoses are in the orientation of the hydroxyl (-OH) groups. Mannose is different from glucose in its configuration at carbon atom 2. Sorbose differs from fructose in its configuration at carbon atom 5. The above formulae of glucose and other sugars show the free carbonyl group and four optically active (asymmetric) secondary hydroxyls. This results in stereoisomerism in sugars. Sugars are designated as D or L. The designation of D or L refers to the two series of sugars, in the D series, the highest number asymmetric carbon has the OH group directed to the right and in the L series this hydroxyl points to the left. The following structures (Figure 3.2) of the simplest sugars, D and L glyceraldehydes explains the series.

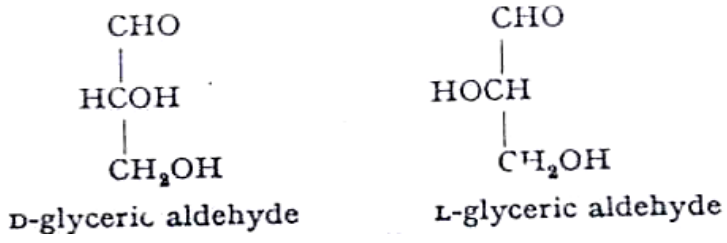

Figure 3.2: D and L glyceraldehydes

Most natural sugars are members of the D series. The open chain formulae of sugars do not explain all the chemical properties. Therefore, various ring structures have been introduced. For example glucose has been assigned a pyranose (6 membered ring) structure and fructose a furanose (5 membered ring) structure.

The simple sugars exhibit a property called optical rotation. You will be learning more on this aspect under polarimetry. In simple terms, optical rotation refers to the property of a substance in solution to rotate plain polarised light to right (dextro rotatory designated by (+)) or to the left (levo rotatory designated by (-)) for example glucose is dextro rotatory and fructose is levo rotatory. A sugar, for example glucose when dissolved in water exist in tautomeric equilibrium between two anomeric forms, each having different degree of optical rotatory power. They are designated as α and β as indicated below (Figure 3.3).

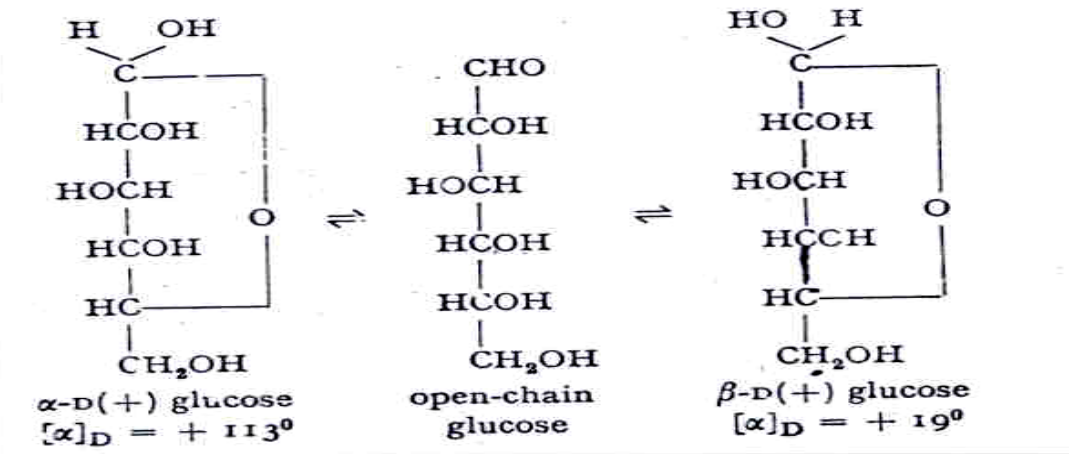

Figure 3.3: α-D and β-D glucose structures

Sugar related compounds present in nature

Amino sugars: Amino sugars usually contain D-glucosamine (2-deoxy-2-amino glucose). They occur as components of high molecular weight compounds such as the chintins of crustaceans and molluscs as well as in certain mushrooms and combined with the ovomucin of egg white.

Glycosides: Glycosides are sugars combined with an alkyl or aryl group. Glycosides are hydrolysed by acid or enzyme to the corresponding sugar and aglicone. Amygdalin is an example of a cyanogenic glycoside, which is present in bitter almonds. Complete hydrolysis of amygdalin yields glucose, benzaldehyde and hydrocyanic acid. Other important glycosides are flavonone glycosides like the citrus bitter principles hesparidin and naringin. Deoxy sugars occur as components of nucleotides like 2-deoxy ribose, which constitute part of deoxy ribo nucleic acid (DNA).

Sugar alcohols: Sugar alcohols occur in some fruits and are produced industrially by reduction of sugars. Reduction of glucose yields sorbitol. Xylitol is a five carbon sugar alcohol. These sugar alcohols are sweet as sugar but are only slowly absorbed in the body and hence are used in diabetic foods. The structure of sorbitol and xylitol are given below (Figure 3.4).

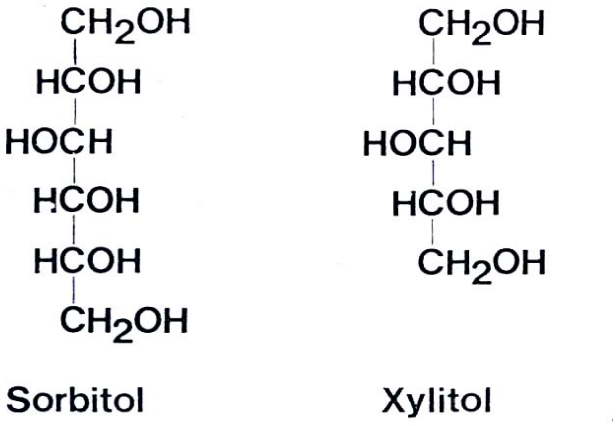

Figure 3.4: Sorbitol and Xylitol

Oligosaccharides

Oligosaccharides are water-soluble polymers of a few condensed monosaccharides. Those most commonly found in foods are homopolymers of D-glucose (e.g. maltose) or heteropolymers of D-glucose condensed with D-fructose (e.g. Sucrose) or D-glucose with D-galactose (e.g. lactose). The above oligosaccharides are di-saccharides. Raffinose a trisaccharide is found in sugar beet. It is a polymer of D-glucose, D-fructose and D-galactose.

Sugars and sweeteners: Sucrose is the most widely used natural sweetener. Of all the sugars, D-fructose is known to be the sweetest. It is customary to compare the degree of sweetness of different sweeteners to sucrose to which the number 100 has been assigned. Fructose has sweetness value of 173.3 and glucose 74.3. Therefore, the hydrolysis product of sucrose (invert sugar containing glucose and fructose) has sweetness value of 173.3+74.3/2 = 123.8. The relative sweetness of various sweeteners is given in Table 3.2.

Table 3.2: Degree of sweetness of various sweeteners

Sweetener	Degree of Sweetness
Sucrose	100
Fructose	173.3
Glucose	74.3
Corn Syrup	30.0
Honey	97.0
Saccharin	30,000 – 50,000
Dulcin	20,000

Corn sweeteners: Cornstarch can be hydrolysed by acid or acid-enzyme process to yield smaller and smaller fragments and ultimately glucose (dextrose). The degree of depolymerisation is expressed as dextrose equivalent (D.E.) which is defined as the amount of total reducing sugars expressed as dextrose and calculated as a percentage of the total dry matter.

Glucose syrup is a concentrated solution of sugars obtained by hydrolysis of starch and having D.E. of 20 or more. When a product has a D.E. of less than 20, it is called maltodextrins. Glucose can be isomerised to fructose by an enzyme called isomerase. As fructose is sweeter than glucose, High Fructose

Syrup (HFS) which is sweeter than glucose syrup is prepared using the enzyme.

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 monosaccharides, oligosaccarides and polysaccarides.

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2. Give three examples of hexoses.

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3. Explain optical rotation.

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4. Give two examples of glycosides.

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5. Give two examples of disaccarides.

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6. What is invert sugar? Why invert sugar is sweeter than sucrose?

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Polysaccharides

Polysaccharides are polymers of the simple mannoses, hexoses or pentoses, which occur in nature in different forms. Unlike the sugars they are not sweet

and are mostly insoluble in water. Examples of polysaccharides found in nature are cellulose, starch, pectin and chitin.

Cellulose: The structural materials of the entire plant world consist largely of cellulose. Cotton is essentially pure cellulose. On hydrolysis cellulose yields glucose meaning that it is a polymer of glucose. Celluloses from different sources have molecular weight ranging from 100,000 to 2,000,000.

Cellulose is insoluble in water and most solvents and is relatively resistant to hydrolysis by dilute acids. Man and carnivorous animals are unable to digest cellulose, since they lack the necessary enzymes for its breakdown in their intestines. However, many microorganisms and protozoa are able to break it down. Ruminants are able to digest cellulose because of the presence of these microorganisms in their intestines. Though cellulose has only indirect food value (cellulose feed is converted to meat by animals) it has many other uses like in the manufacture of paper, textiles, explosives, paint, etc.

Starches: Starch is the most important polysaccharide and is distributed widely in nature as a reserve material in plants. It contributes more calories to the normal human diet than any other single nutrient.

Starch from different sources consists of granules of different shapes and sizes. Generally all starches contain two types of molecular structures namely amylose and amylopectin.

Amylose is a long straight chain of glucose units, which reacts with iodine to give blue colour. Amylopectin is a branched molecule consisting of a number of amyloses, which reacts with iodine to give reddish brown colour.

Amylose and amylopectin content of starches from different sources vary. In the most common starches such as corn, rice and potato, amylose is the minor component and represents about 17 to 30% of the total. Some varieties of pea and corn starch may have as much as 75% of amylose.

Starch granules naturally present in plants are completely insoluble in cold water and upon heating they will start to swell and the gelatinisation temperature. Continuation of heating above this temperature results in further swelling of the granule and the mixture becomes viscous and translucent. When such a paste is agitated, the swollen starch granule structure breaks down and the viscosity greatly reduces. When a cooked starch paste is cooled, it may form a gel or under conditions of slow cooling, the linear compound may form a precipitate. This phenomenon called retrogradation. The staling of bread is ascribed to retrogradation of starch. The rate of staling is temperature dependent. Retrogradation is faster at low temperature and hence bread stales more quickly in the refrigerator than at room temperature. Freezing, however, prevents staling and retrogradation of starch.

The functional properties of starches such as its cooked paste viscosity play an important role in their food applications. Cereal starches (corn, wheat, rice and sorghum) form viscous short bodied pastes which set to opaque gels on cooling. Root and tuber starches (potato, cassava or tapioca) form highly viscous paste, which are clear but on cooling become weak gels. Waxy starches (waxy corn and rice) form heavy bodied springy pastes. These pastes

are clear and have a low tendency for gel formation. High amylose starch requires high temperature for gelatinisation and gives short bodied paste which form very firm opaque gels on cooling. These are suitable for film formation, which find application in coating of fruits as edible coats. In order to impart the desired functioning properties, starches can be modified by certain chemical or enzymes treatments. Such starches are called 'modified starches'.

Pectic substances: Pectic substances occur as constituents of cell walls. In the native form, they are bound to cellulose as protopectin. Mild acid hydrolysis of protopectin yields pectin or pectinic acid. Pectinic acids are collidal, galacturonic acids containing more than negligible proportion of methyl ester groups. Pectins are water dispersable pectinic acids having varying methyl ester contents that are capable of forming gels with sugar and acid under suitable conditions. Pectic acids are composed mostly of colloidal polygalacturonic acids and are essentially free from methyl ester groups.

A linear chain of anhydro-D-galacturonic acid units is the basic structure of pectic substances. In an earlier section you have studied the structure of galactose. When the –CH2OH of a hexose is replaced by –COOH it becomes an uronic acid. The chemical structures of some uronic acids are shown in Figure 3.5.

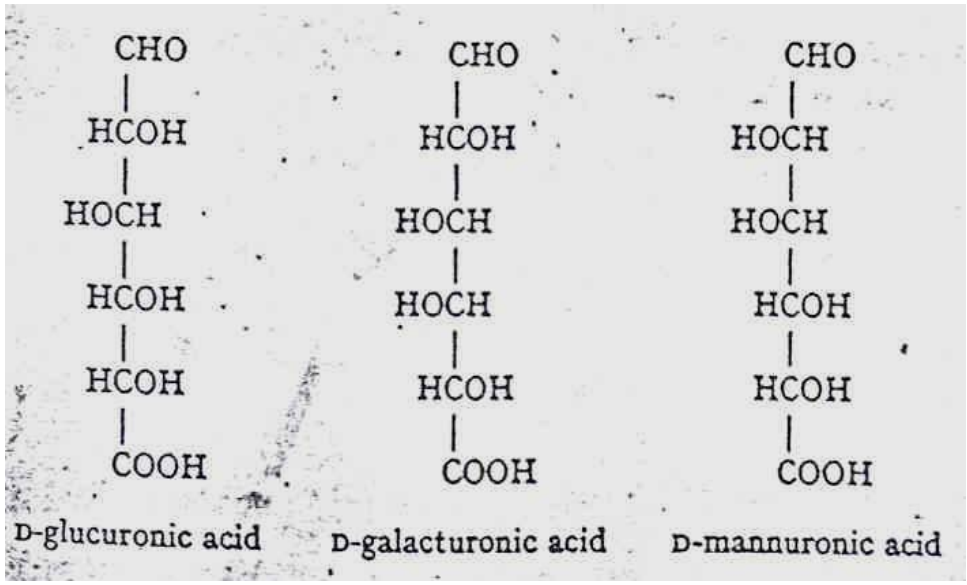

Figure 3.5: Uronic acids

A number of enzymes have been found to catalyse the various stages of pectin break down. You are familiar with softening of fruits during their ripening. It is due to the break down of pectin by the pectin degrading enzymes present in the fruits. There are two major types of pectin degrading enzymes. One is called pectin esterase (PE) or pectin methyl esterase (PME). This enzyme removes the methoxyl groups from the pectin molecules thereby reducing the viscosity of pectin solution and destroys its gelling property. The other enzyme is called polygalacturonase (PG), which breaks down pectinic acid chain into smaller fragments. For PG to act on pectin, the pectin molecule has to be initially demethylated to pectic acid by PME

Pectin as a jellifying agent: The most important use of pectin in food is based on its ability to form gels and it is therefore widely used in the manufacture of jams, jellies and marmalades. Depending on the degree (extent) of methylation (DM) of the carboxyl groups in pectin, they are classified into high and low methoxyl pectins (HMP and LMP). Generally, pectin having more than 50% methylated carboxyl groups (DM more than 50) are called high methoxyl pectin (HMP). HM pectins form gels with sugar and acid. For a good jelly, the sugar content should be in the range of 65–70% and pH 2.8 to 3.2. When the degree of methylation is below 50, the pectin is called low methoxyl pectin (LMP). Low methoxyl pectins form gels at a lower level of sugar but with divalent cations especially calcium. They can be gelled over a much wider pH range also. Therefore, LMP is used in preparing low sugar diabetic jams and jellies.

Citrus peel and pomace as well as apple pomace are good sources of pectin. Therefore, these raw materials are usually used for the manufacture of pectin.

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 the difference between cellulose and starch?

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2. What is the basic molecule in pectin?

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3. What is the difference between high and low methoxyl pectins?

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Gums: The term gum has been applied to many substances, both hydrophilic (water attracting) and hydrophobic (water repelling) that has gummy characteristics. Sometimes hydrophobic gums are called resins. Gums are essentially polysaccharides and are of plant and microbial origin.

Plant gums: Plant gums can be classified as seed gums, plant exudate gums and seaweed gums. They find wide food applications as stabilisers, thickeners, foam stabilisers, clarifying agents, flavour fixing agents, etc. Table 3.3 gives details of some vegetable gums used as food additives.

Table 3.3: Properties and use of some vegetable gums

Name and Source	Properties
Seed gums	
Guar gum *(Cyamopsis tetra gonoloba)*	Non ionic, heat stable, hydrates in cold water
Locust bean gum *(Ceratonia siliqua)*	Like guar but heat required for maximum hydration and viscosity.
Plant exudate gums	
Gum Arabic *(Acacia senegal)*	Highly soluble, low viscosity, clear solution.
Seeweed extracts	
Agar *(Rhodophyceae)*	Forms strongest and most stable gel. Gels are transparent and reversible upon heating and cooling.
Carrageenans *(Rhodophyceae)*	Anionic, forms stable complexes with proteins and other gums.
Alginates *(Phaeophyceae)*	Anionic, widely variable viscosity properties in acid and salt, forms gels and films

Microbial gums: Polysaccharides produced by microorganisms are finding wide food applications today. Dextran gums are produced by the action of micro- organisms like *Leuconostoc mesenteroides* on sugar syrup. They are readily soluble in water and have typical characteristics of a hydrocolloid like plant gums. Xanthan gum is produced by the action of *Xanthomonas campestris* on sugar containing medium. This gum consists of condensed D-glucose, D-mannose and D-glucuronic acid groups.

3.2.3 Chemical Reactions of Carbohydrates

Several of the chemical reactions of carbohydrates affect the food quality. During food processing operations, especially heat processing like sterilisation, cooking and dehydration, carbohydrates undergo several changes. Part of the non reducing sugars (like oligosaccharides) and polysaccharides hydrolyse to form reducing sugars. The carbonyl groups of the reducing sugars combine with the basic amino groups of proteins, peptides and amino acids to form sugar – amine compounds which undergo a series of reactions called Maillard reactions to produce dark coloured compounds and sometimes off flavour. This reaction is also referred to as non-enzymatic browning reaction to distinguish the enzyme mediated browning we observe, for example in cut fruits like apple where it is oxidation of some phenolic compounds.

Sugars alone in the absence pf amino acids can also cause browning or blackening. At very high temperatures (above 100°C) reducing sugars condense with each other to form higher oligosaccharides and cyclic compounds. Such darkening reaction of sugars is called caramalisation.

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. Give three examples of plant gums.

   ……

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   ……

2. Explain microbial gums.

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   ……

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   ……

3. Explain Maillard reaction.

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3.3 LIPIDS

Of the three most important nutrients, carbohydrates, proteins and edible fats, the latter belongs to a large class of very diverse substances called lipids. They can be classified into three main groups, their common property being that all contain fatty acids.

3.3.1 Occurrence and Classification

a) Fats and oils: They consist of triglycerol esters of fatty acids (designated here as F1, F2, F3) of the following general formula (Figure 3.6), which easily undergo hydrolysis forming glycerol and fatty acids.

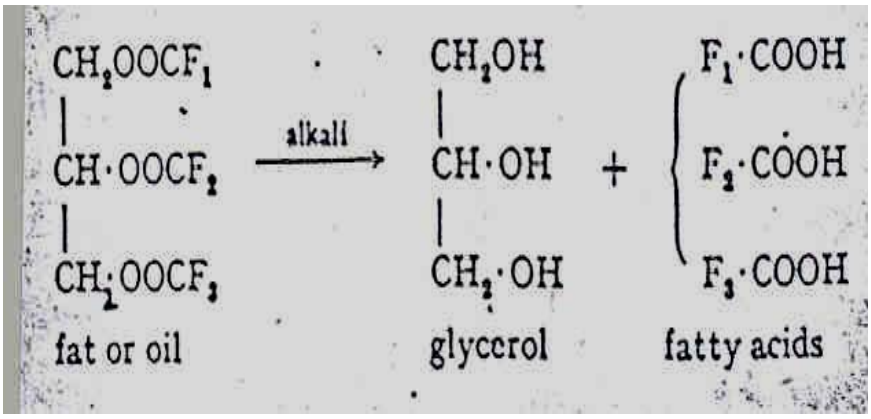

Figure 3.6: Alkali hydrolysis of fats and oils

Liquid fats are commonly referred to as oils.

b) Waxes: They consist of fatty acids esterified by monohydric long chain alcohols such as melyssil alcohol (C30H61OH) in bees wax.

c) Phospholipids: They are complex compounds in which glycerol or other alcohols are esterified partly by fatty acids and partly by phosphoric acid and by basic nitrogen compounds.

Lipids occur in all parts of plants and animal tissues. However, they are abundantly found in specific fat tissues, seeds and nuts (Table 3.4).

Table 3.4: Fat content of some foods

Food	Fat (%)
Cereals	
Maize	3.6
Rice	0.6
Wheat	1.5
Wheat germ	7.4
Pulses	
Bengal gram	5.3
Black gram	1.4
Green gram	1.3
Peas, dried	1.1
Soyabean	19.5
Leafy vegetables	
Amaranth	0.5
Cabbage	0.1
Drumstick leaves	1.1
Nuts	
Almond	58.9
Groundnut	40.1
Sesame seed	43.3
Fruits	0.1
Apple	22.4
Avocado (Butter fruit)	

Seafoods	
Bombay duck	0.7
Sardine (High fat)	14.3
Meat	
Poultry meat	0.6
Mutton	13.3
Egg (hen)	13.3
Dairy products	
Milk (cow)	3.6
Milk (buffalo)	8.8

3.3.2 Fatty Acids

Even number straight chain saturated and unsaturated fatty acids makeup the greatest portion of the fatty acids of the natural fats. The fatty acid composition of fats and oils has great bearing on human health.

Saturated fatty acids: These are straight chain acids with an even number of carbon atoms from C_2 to $C_{26.}$ Their general formula is:

$$CH_2 (CH_2)_n COOH$$

Where n is the number of – CH_2 groups.

The most widely distributed fatty acids in oils and fats are palmitic, lauric and stearic. It is customary to designate fatty acids by the number of carbon atoms they have. For example 16:0 refers to a fatty acid with 16 carbon atoms and zero refers to the number of double bonds (unsaturated carbon atoms). The chemical formulae of the three fatty acids are given below:

Lauric acid (12:0) : $CH_3. (CH_2)_{10}.COOH$
Palmitic acid (16:0) : $CH_3.(CH_2)_{14}. COOH$
Stearic acid (18:0) : $CH_3 (CH_2)_{16.} COOH$

Unsaturated fatty acids: The most widely distributed unsaturated fatty acids in oils and fats are given below:

Oleic acid (18:1) : CH3.(CH2)7 CH=CH.(CH2)7. COOH
Linoleic acid (18:2) : CH3.(CH2)4 (CH:CH.CH2)2. (CH2) 6.COOH
Linolenic acid (18:3) : CH3.CH2. (CH: CH.CH2) 3. (CH2) 6.COOH
Archadonic acid (20:4) : CH3.(CH2)4. (CH: CH.CH2) 4. (CH2)2.COOH

3.3.3 Properties of Fats and Oils

Lipids are water insoluble but soluble in organic solvents such as petroleum ether, hexane, chloroform etc. Certain physical and chemical properties of fats and oils help in identifying them. Fats do not melt sharply but soften over a range of temperatures and therefore melting point is not a very reliable technique to identify a fat. However, it provides some information on its identity. Different oils and fats have refractive indices with narrow variations. Therefore, refractive index measurement helps in testing the purity. Their smoke point, flash point and fire point characterizes fats and oils. The smoke point is the temperature at which a fat or oil gives off a thin bluish flame. The

flash point is the temperature at which the mixture of vapour with air will ignite and the fire point is the temperature at which the substance will sustain continued combustion.

Chemical properties: A number of chemical tests have been evolved to identify fats and oils and to detect adulteration. All oils and fats show some range of values and therefore sometimes more than one test is necessary.

Reichert–Meissl number: It is a measure of the amount of water soluble volatile fatty acids. It is defined as the millilitres of 0.1N alkali (such as potassium hydroxide) required to neutralise the volatile water soluble fatty acids in a 5g sample of fat. The common volatile water soluble fatty acids are butyric (C4) and caproic (C6) and caprylic (C8) acids. Reichert-Meissl number is particularly valuable in detecting adulteration in butter.

Saponifaction number: It is defined as the number of milligrams of potassium hydroxide required to saponify one gram of fat or oil. When potassium hydroxide reacts with a triglyceride, 3 moles of potassium hydroxide react with one molecule of fat. If the triglycerides contain low molecular weight fatty acids, the number of molecules present in 1 gram sample of fat will be greater than if the fatty acids have long carbon chains and higher molecular weights. The fats with the low molecular weight fatty acids will consequently have a higher saponifaction number. For example, butter with its unusually high percentage of butyric acid has the highest saponification number.

Iodine number: It is the number of grams of iodine absorbed by 100 g of fat. The double bonds found in the unsaturated fatty acid react readily with iodine or certain iodine compounds to form an addition compound even while the fatty acid is combined with glycerol in fat. The iodine number is therefore, a measure of the extent of unsaturation of the fatty acids in a fat.

Rancidity: We are familiar with the development of rancid smell in oils on storage. The process of auto oxidation and the resulting deterioration in flavour of fats and fatty acids are often described by the term ‘rancidity’. Temperature, moisture, the amount of air in contact with the oil or fat, light as well as the presence or absence of antioxidants influence rancidity development. Usually rancidity is referred to oxidative deterioration, but in many fats especially butter, rancidity refers usually to hydrolytic changes resulting from enzymatic activity. In general animal fats develop rancidity faster than vegetable or seed fats.

Oxidation of fats and oils is usually referred to as auto oxidation, because the rate of oxidation increases as the reaction proceeds. Fats and oils containing unsaturated fatty acids are generally susceptible for auto oxidation, though in practice deviations are possible due to the presence of natural antioxidants in them. The reaction products of auto oxidation of oils and fats are peroxides, hydroperoxides, aldehydes and short chain fatty acids which are responsible for the off flavour. **Peroxide value** of fats measures the extent of rancidity development. It is based on the amount of iodine released from potassium iodide by peroxides.

Lypolysis: Fats and oils also become rancid due to lypolysis. The ester linkages of lipids are subject to hydrolysis resulting from enzymes, heat, or chemical

reactions. These reactions are collectively known as lypolysis, lypolytic rancidity or hydrolytic rancidity. The free fatty acids that develop during storage and processing of oil seeds and animal tissues must be removed by a refining process. The lower free fatty acids being volatile by steam can be removed by steam distillation under vacuum while the remaining fatty acids are converted by means of sodium or potassium hydroxides into soaps by settling or centrifugation.

Antioxidants: Antioxidants are nothing more than substance with preferential ability to oxidize namely certain compounds, which will oxidize prior to the substances that are being protected. They are both water soluble and fat soluble. For protecting fats, oils and fatty foods, fat-soluble antioxidants are required. Butylated hydroxy anisole (BHA), Butylated hydroxy toluene (BHT) and esters of gallic acid are common antioxidants for this category of foods. Many naturally occurring substances also function as antioxidants. Most prominent are tocopherols. Their presence in natural vegetable oils is the cause for stability of such oils.

Hydrogenation: The physical requirement of many fats used in foods is generally different from those of natural fats and oils. Hydrogenation, the direct addition of hydrogen to double bonds of fatty acids is used to modify vast quantities of fats and oils. Vanaspati is an example of hydrogenated fat. Close control of hydrogenation results in highly specific results. For example, salad and cooking oils can be improved by controlled hydrogenation. Hydrogenation of fats and oils is achieved by mixing them with hydrogen at elevated temperature in the presence of a suitable catalyst, the most common being nickel.

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. Give an example of a fruit containing high level of fat.

   …….

   …….

   …….

2. What are the hydrolysis products of fats and oils?

   …….

   …….

   …….

3. What is the difference between saturated and unsaturated fatty acids? Give two examples for each.

   …….

   …….

   …….

4. Define Reichert-Meissl number and iodine number of fats and oils.

……

……

……

3.4 LET US SUM UP

Carbohydrates are widely distributed in nature. They are grossly classified into monosaccharides, oligosaccharides and polysaccharides. Simple sugars like glucose and fructose are monosaccharides. Sucrose (cane sugar) is the most important disaccharide.

Unlike monosaccharides and oligosaccharides, polysaccharides like cellulose and starches are insoluble in water. Both cellulose and starches are made up of glucose units, but differ in their number of glucose units and their nature of bonding.

Sugars undergo browning reactions during processing and storing. Nonenzymatic browning reactions (Maillard reactions) are initiated mainly due to the reaction between reducing sugars and their amino acids.

Plant gums are also a class of polysaccharides. They have many food and industrial applications.

Lipids are distributed widely in plant and animal foods. They are classified into oils and fats, waxes and phospholipids. Oils and fats on hydrolysis yield fatty acids and glycerol. Fatty acids can be saturated or unsaturated. There are different methods to find out the degree of unsaturation in fats and oils. Unsaturated fatty acids in fats and oils are responsible for rancidity development. Adding antioxidants to fats and oils and fatty foods can prevent development of rancidity.

3.5 KEY WORDS

Monosaccharides : Simple sugars or monoses, readily soluble in water and most of them are sweet to taste.

Hexoses : Six carbon sugars like glucose and fructose.

Oligosaccharides : Water soluble polymers of a few condensed monosaccharides.

Disaccharides : Polymers of two condensed monosaccharides like sucrose, maltose and lactose.

Degree of sweetness (DS) : Sweetness scale in which sweetness of sucrose is assigned the number 100. Fructose has DS of 173.3 and saccharin 30,000 to 50,000.

Polysaccharides : Polymers of simple sugars having high molecular weights. Examples are cellulose and starch.

Pectic acid : Unmethylated poly galacturonic acid.

High and low methoxyl pectins : Pectic acid in which more than 50% of the carboxyl groups are methylated and in which less than 50% carboxyl groups methylated are called high and low methoxyl pectins respectively.

Gums : Complex polysaccharides of plant and microbial origin having gummy characteristics.

Maillard reaction : Reaction between reducing sugars and amino acids leading to brown compounds.

Rancidity : Development of rancid odour in oils and fatty foods on storage.

3.6 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

Your answers should include the following points:

1. Simple sugars, polymers of few monomers, water soluble, sweet. Polymers of simple sugars of large molecular weight.
2. Glucose, fructose, mannose, sorbose.
3. Rotation of plain polarised light.
4. Amygdaline, naringin.
5. Sucrose, maltose, lactose.
6. Sucrose hydrolysis, fructose sweeter than glucose.

Check Your Progress Exercise 2

Your answers should include the following points:

1. α and β linkages, degree of polymerisation.
2. Galacturonic acid
3. Degree of methylation of pectic acid.

Check Your Progress Exercise 3

Your answers should include the following points:

1. Guar gum, gum Arabic, carrageenans.
2. Xanthan, dextran.
3. Sugar – amino acid reaction.

Check Your Progress Exercise 4

Your answers should include the following points:

1. Avocado
2. Glycerol and fatty acids
3. Double bonds, palmitic, stearic, oleic, linolenic
4. Water soluble volatile fatty acids
5. Degree of unsaturation
6. Oxidation of unsaturated fatty acids

3.7 SOME USEFUL BOOKS

1. Owen R. Fennema, (1976) Principles of food science, Part I-Food Chemistry, Marcel Decker Inc.; New York.

2. Meyer L.H. (1969) Food Chemistry, Van Nostrand Reinhold Company, New York, Cincinnati, Toronto, London, Melbourne.

3. Braverman, J.B.S. (1963) Introduction to the Biochemistry of foods, Elsevier Publishing Company, Amsterdam, London, New York.

UNIT 4 FOOD CONSTITUENTS – PROTEINS, ENZYMES AND WATER

Structure

4.0 OBJECTIVES

After reading this unit, you will be able to answer:

- what are proteins and amino acids? How proteins are classified? Protein structure, their Denaturation and the role of protein and amino acids in non-enzymatic browning. Proteins of plant, seeds, marine and animal origin. Classification and properties of enzymes etc.; and
- you will also learn the importance of the state of water in foods in food preservation, freezing of water, water quality and standards and water analysis.

4.1 INTRODUCTION

Unlike the other two major nutrients viz., carbohydrates and lipids that are essentially energy sources, proteins constitute the main structure of the animal and human body. These constituents characterised by their nitrogen content are involved in many vital processes intricately associated with all living matter. Some proteins function as biocatalysts (enzymes). There are different types of enzymes in all living systems. They catalyse most of the biological reactions. As enzymes have high degree of specificity, mostly one enzyme can catalyse only one reaction. Several enzymes like amylase, invertase, glucose oxidase, Pectinases, proteases, find application in food processing.

Water is an essential constituent of foods. The state of water in foods has great bearing on food preservation. The physical, chemical and microbiological quality of water used in food processing operations should conform to certain minimum standards.

4.2 PROTEINS

Protein is one of the three major basic nutrients required for growth and development, the other two being carbohydrates and lipids. The word protein was coined from the Greek proteios, which means 'of the first rank'. Proteins are very complex organic substances, constitute the main structure of the animal and human body. These macromolecules, characterized by their nitrogen contents are involved in many vital processes intricately associated with all living matter. Some proteins function as biocatalysts (enzymes) and hormones to regulate chemical reactions within the body.

4.2.1 Amino Acids

Amino acids are the building blocks of proteins. Proteins are polymers of some 20 amino acids joined together in different proportions and sequences. Most amino acids have general chemical structure as given in Figure 4.1.

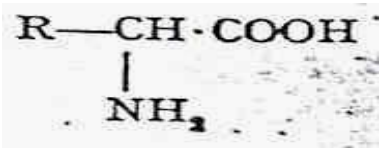

Figure 4.1: General chemical structure of amino acids where R= aliphatic, aromatic, heterocyclic etc. groups

They have both amino group and acidic carboxyl group. In proteins the amino acids are joined together by peptide bond (-CO-NH-) (Fig.2) i.e. The carboxyl group of one amino acid is linked with the amino group of the second amino acid with elimination of H_2O.

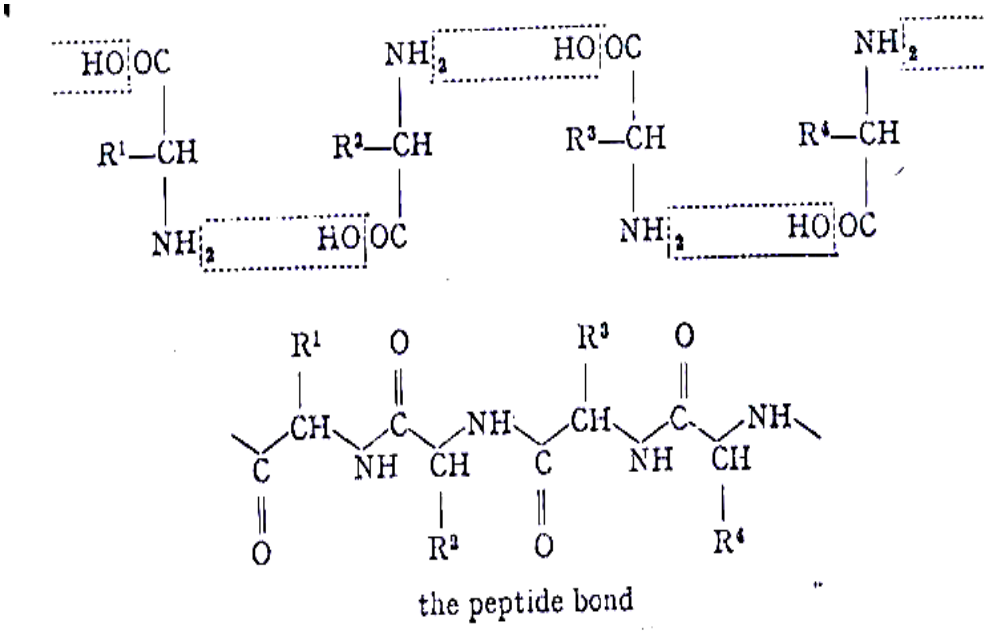

Figure 4.2: The peptide bond

Amino acids found in nature are classified into six groups: viz.,

1. Aliphatic mono amino mono carboxylic amino acids e.g. glycine, alanine, valine, leucine, isoleucine, serine and threonine.

2. Sulphur containing amino acids e.g. cysteine, cystine and methionine.

3. Aliphatic mono amino dicarboxylic amino acids, e.g. aspartic acid and glutamic acid.

4. Aliphatic basic amino acids, e.g. lysine, arginine and histidine.

5. Aromatic amino acids, e.g. phenylalanine and tyrosine.

6. Heterocyclic amino acids, e.g. triptophan and proline.

Of the above amino acids, eight for adults and ten for children are considered essential or indispensable for the human diet. They are: lysine, triptophan, phenylalanine, threonine, valine, methionine, leucine and isoleucine. The amount of these amino acids present in a protein and their availability determine the nutritional quality of the protein. In general animal proteins are of higher quality than plant proteins. However, plant proteins can be upgraded nutritionally by judicious blending. That is why, traditionally people consume a variety of pulses. Egg protein is one of the best quality proteins and is considered to have a biological value of 100. It is widely used as a standard and protein efficiency ratio (PER) values are sometimes based on egg white as a standard. Cereal proteins are generally deficient in lysine and threonine. Soya is a good source of lysine but deficient in methionine. Cottonseed protein is deficient in lysine and groundnut protein in methionine and lysine. The protein of potato although present in small quantity is of excellent quality and is equivalent to that of whole egg.

4.2.2 Protein Classification

Proteins are divided into two main groups namely simple and conjugated and derived proteins.

Simple Proteins

Simple proteins yield only amino acids on hydrolysis and include the following classes:

a) Albumins: They are soluble in neutral salt free water. Usually these are proteins of relatively low molecular weights. E.g. egg albumin, lactalbumin and serum albumin in the whey proteins of milk, leucosin of cereals, legumelin in legumes.

b) Globulins: They are soluble in salt solutions and almost insoluble in water. E.g. Serum globulins and β-lacto globulin in milk, myosin and actin in meat, glycinine in soybean.

c) Glutelins: Soluble in very dilute acids or bases, insoluble in neutral solvents. E.g. Wheat glutelin and oryzenin in rice.

d) Prolamins: Soluble in 50-80% ethanol and insoluble in water. E.g. Gliadin in wheat, zein in corn and hordein in barley.

e) Scleroproteins: Insoluble in water and neutral solvents and resistant to enzymatic hydrolysis. These are fibrous proteins serving structural and binding purposes. Gollagen of muscle tissue, elastin of tendens, creatin of hair and fibroin of silk are examples.

f) Histones: Basic proteins containing a large number of basic amino acids like lysine and arginine. Soluble in water and precipitated by ammonia.

g) Protamins: Strongly basic proteins of low molecular weights. They are rich in arginine. E.g. Cupein from herring, and scombrin from mackerel.

Conjugated Proteins

Conjugated proteins contain an amino acid part combined with a non protein material such as lipids, nucleic acid, carbohydrates and others. Some of the conjugated proteins are:

1. *Phospho proteins:* They constitute an important group including many major food proteins. This group includes casein of milk and the phosphoprotein of egg yolk.

2. *Lipoproteins:* These are combination of lipids with proteins and have excellent emulsifying capacity. Lipo protein occurs in milk and egg yolk.

3. *Nucleoproteins:* These are combination of nucleic acids with protein. They are found in cell nuclei.

4. *Glycoproteins:* These are combination of carbohydrates with protein. Ovomucine of egg white is an example.

5. *Chromo protein:* These are proteins with coloured prosthetic groups. Hemoglobin, myoglobin, chlorophyll and flavo proteins are examples.

4.2.3 Protein Structure

The Primary structure of proteins is related to the peptide bonds between the component amino acids and also to the amino acid sequence in the molecule. A peptide chain may become involved in hydrogen bonding between amide nitrogen and carbonyl oxygen. These bonds may be formed between different areas of the same polypeptide chain or between adjacent chains. Such bonds establish the secondary structure of proteins, which may be of helical or sheet form.

The tertiary structure of protein is established when the chain are folded into compact structures stabilized by hydrogen bonds, disulphide bridges, etc. Large molecules may form quaternary structures by association of sub units.

4.2.4 Protein Denaturation

Denaturation is a process of change in structure of proteins without breaking covalent bonds. The process is peculiar to proteins and affects different proteins to different degrees. Denaturation can be brought about by a variety of agents of which the most important are heat, pH, salts and surface effects. The destruction of enzyme activity by heat is one of the most important operations in food processing. You are familiar with the coagulation or hardening of egg white on heating it. It is due to denaturation of egg albumin. Freezing can also cause protein denaturation as in the case of fish, which becomes tough on freezing and thawing. Milk protein casein and gelatin are examples of proteins, which can be boiled with out apparent denaturation.

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. Name the different groups of amino acids.

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   ……

   ……

2. Define albumins and globulins.

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3. What are phosphoproteins and lipoproteins?

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4.2.5 Non-Enzymatic Browning

Browning of several foods is familiar to all of us. Browning of potato chips, brown crust formation on bread and cakes, browning of evaporated milk, browning of jams, candies, fruit juice concentrates etc. are all examples of non-enzymatic browning. The browning reaction can be defined as the sequence of events, which begins with the reaction of the amino group of amino acids, peptides or proteins with a glycosidic hydroxyl group of sugars and terminates with the formation of brown nitrogenous polymers or melanoidins. While some browning reactions like brown crust formation on bread and cake are desirable,

most others are undesirable and may accompany formation of off flavoured compounds.

Methods of preventing browning could consist of measures indented to slow the reaction rates such as control of moisture, temperature or pH or removal of an active intermediate. Generally it is easy to use an inhibitor. One of the most effective inhibitors of browning is sulphur dioxide. It is known that sulphur dioxide can combine with the carbonyl group of an aldose to give an addition compound thus blocking further transformations leading to formation of dark coloured compounds.

$$NaHSO_3 + R\text{-}CHO \rightarrow RCHOHSO_3Na$$

However, as sulphite can destroy the vitamin thiamine, it is not desirable to use it to inhibit browning in foods, which are good sources of this vitamin.

4.2.6 Protein from Different Sources

Human requirement of proteins is met from both animal and plant sources.

Proteins of Animal Origin

A typical adult mammalian muscle contains 18-20% protein. Muscle proteins are categorized on the basis of their origin and solubility as sarcoplasmic, myofibrillar and stroma proteins. Protein content of milk ranges from 3 to 4%. Buffalo milk has slightly higher level of protein. Milk proteins are grossly divided into casein and whey protein, the average ratio being 80:20. Egg contains on an average 11% shell, 31% yolk and 58% white. The yolk contains about 50% solids of which one third is protein and two third lipids. Egg white is essentially an aqueous solution containing about 12% protein.

Proteins of Marine Origin

Fish flesh contains on an average 10-21% protein. Fish muscle proteins, like those of mammalian muscle are generally classified as sarcoplasmic, myofibrillar and stroma proteins but their proportions differ.

Proteins of Plant Origin

The protein source of vegetarian diet is from cereals, pulses and oil seeds besides small quantities from vegetables.

Cereals like wheat and rice are important sources of protein because they are the staple foods of Indians. On an average wheat has 12-13% protein while rice has 7-9% protein. Gluten proteins are responsible for the unique bread making property of wheat. Wheat and rice proteins are generally deficient in the essential amino acid lysine.

Seed Proteins

Legumes (pulses) and oil seeds are major sources of vegetable proteins. The average protein content of the major pulses is given in Table 4.1.

Table 4.1: Protein content of major pulses

Legume	Protein (%)
Bengal gram dhal	20.8
Black gram dhal	24.6
Field bean, dry	24.9
Green gram dhal	24.5
Lentil (lens culinaris Medic)	25.1
Peas, dried	19.7

The average protein content of some of the oil seeds is given in Table 4.2.

Table 4.2: Protein content of some oil seeds

Oilseed	Protein (%)
Ground nut	26.7
Soybean	43.2
Sesame	18.3
Cotton seed	19.5
Sunflower seed	12.5

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. Explain protein denaturation.

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2. What are the major difference between proteins of animals and plants?

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3. Name four good sources of plant proteins.

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4 Which are essential amino acids?

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4.3 ENZYMES

Enzymes are called bio- catalysts. They are globular protein catalysts that accelerate several biological reactions. Enzymes are present in all biological systems both plant and animal. Enzymes show high degree of specificity. This property is very important in food processing where it is often desirable to modify only a single component in the process.

4.3.1 Nomenclature and Classification

Enzymes are classified into six major groups depending on the type of reactions they catalyze i.e. 1) oxido reductases, 2) transferases, 3) hydrolases, 4) lyases, 5) isomerases and 6) ligases

Over the years, thousands of enzymes have been isolated and identified. Quite often two or three enzymes differ only slightly from one another in their properties causing difficulty in naming them. Therefore, the International Union of Biochemistry on Nomenclature and Classification of Enzymes has assigned a code number of four numerals for each enzyme, which fully identifies an enzyme. However it is too cumbersome to follow this classification and therefore for routine purpose most of the enzymes have been given a trivial name, which is short and simple.

4.3.2 Properties of Enzymes

As already mentioned, all enzymes are proteins but all proteins are not enzymes. Some enzymes consist of protein only, but most enzymes contain additional non- protein components such as carbohydrates, lipids, metals, phosphates or some other organic moiety. The complete enzyme is called holoenzyme, the protein part apoenzyme and the non- protein part cofactor. The compound which is being converted in an enzyme reaction is called substrate, In an enzyme reaction the substrate combines with the holoenzyme and is released in a modified form as shown below.

$$\text{Enzyme} + \text{Substrate} \leftrightarrows \text{Complex} \leftrightarrows \text{Enzyme} + \text{Products}$$

Enzyme activity is affected by various factors like temperature, pH, chemicals etc. By far the most important is the requirement of optimum temperature and pH for their maximum activity. These properties serve both for obtaining maximum activity for an enzyme as well as for inhibiting the enzyme activity.

By far the largest group of enzymes important in food processing is the hydrolases. A few oxido reductases and isomerases are also encountered. Hydrolases catalyse the following general reaction.

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

Typical examples of hydrolases are amylases, pectin esterase, poly galacturonase, proteases, lipases etc. Most of the enzymes used in industrial applications are now obtained from microorganisms. Table 4.3 gives examples of some of the important enzymes encountered in food processing.

Table 4.3: Some enzymes used in food processing

Enzyme	Food	Purpose/ action
Amylases	Baked products	Increase sugar content for yeast fermentation
	Brewing	Conversion of starch to maltose for fermentation
Invertase	Artificial honey	Conversion of sucrose to glucose and fructose
Naringinase	Citrus juice	De-bittering of citrus juice
Pectinases	Fruit juice	Improve yield and clarity
Proteases	Brewing	Clarification, chill-proofing
	Meat and fish	Tenderization
Lipases	Oils	Hydrolytic rancidity (deteriorative)
Glucose oxidase	Egg powder	Prevention of browning by removing glucose
Polyphenol oxidase	Fruits	Enzymatic browning (deteriorative)

4.3.3 Immobilized Enzymes

One of the most important recent developments in the use of enzymes in industrial food processing is the fixing of enzymes on water insoluble inert supports. The fixed enzymes retain their activity and can be easily added or removed from the reaction mixture. The use of immobilized enzymes permits continuous processing and repeated use of the enzyme. Another important use of immobilized enzymes is in analytical and medical fields.

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 the role of enzymes?

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2. Are all proteins enzymes?

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3. Explain immobilized enzymes?

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4.4 WATER

Water is an essential constituent of most foods. It may occur as an inter cellular and/or extra cellular component in vegetables and animal products, as a dispersing medium or solvent in a variety of products, as the dispersed phase in some emulsified products such as butter or margarine and as a minor constituent in other cases. The presence of water influences deterioration of food, either chemical or microbiological. Thus an understanding of its properties and its behaviour in foods is of great importance.

4.4.1 State of Water in Foods

Water is present in foods in different forms. They are grossly characterised as bound water and free water. Bound water is the water, which is bound to other constituents of foods like proteins and remains unfrozen. This water is unavailable as a solvent. The state of water in food is described by the relationship between the moisture content of the product and the relative humidity of the air surrounding it. This ratio is called water activity, which is an important characteristic of the system. The relative humidity corresponding to each specific moisture content of the product called equilibrium relative humidity (ERH) and the following relationship applies.

$$a_w = p/p_o = ERH/100$$

where a_w = water activity
p = partial pressure of water in food
p_o = vapour pressure of water at the same temperature
ERH = Equilibrium Relative Humidity in %

At high moisture contents, when the amount of moisture exceeds that of solids, the activity of water is close to or equal to 1.0. When the moisture content is lower than this amount, water activity is lower than 1.0. Below moisture content of about 50% the water activity decreases rapidly and the relationship between water content and relative humidity is represented by the sorption isotherm. The adsorption and desorption processes are not fully reversible;

therefore, a distinction can be made between the adsorption and desorption isotherms according to whether a dry product is subjected to increasing moisture levels or whether the moist product is gradually equilibrated with lower moisture levels and the product is being dried. Figure 4.3 shows the adsorption desorption isotherms.

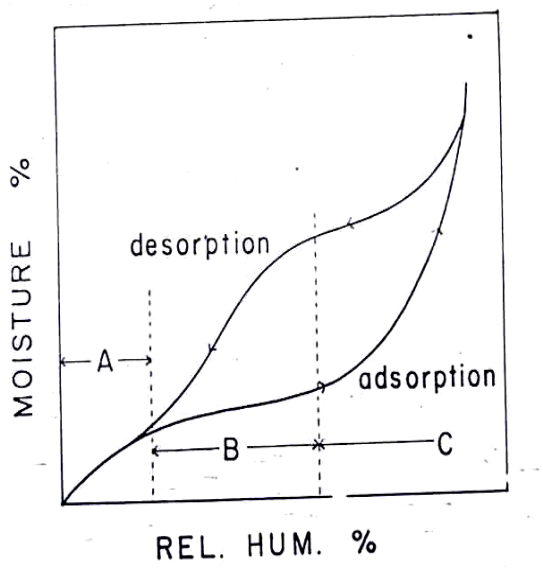

Figure 4.3: Adsorption desorption isotherms

Generally the isotherms are required for the observation of hygroscopic products and the desorption isotherms are useful for investigation of the process of drying. A steeply sloping curve indicates that the material is hygroscopic and a flat curve points to a product, which is not sensitive to moisture.

4.4.2 Water Activity and Food Spoilage

Moisture content and water activity are of major importance in affecting a progress of chemical and microbiological spoilage reactions in foods. Dried and dehydrated foods have very good microbiological stability though browning reactions take place. Such products have moisture content in the range of 5-15% and have low water activity. Intermediate moisture foods (IMF) have moisture content in the range of 20–40% and are fairly shelf stable. IMFs generally have water activity above 0.5. Adding/having soluble solids like sugar achieve this. Example of such products is jams, preserves, cakes, dry fruits, etc. Bacterial growth is practically nil below water activity of 0.90. Yeasts and moulds are usually inhibited between 0.88 and 0.80 water activity, although some osmophilic yeast can tolerate water activity as low as 0.65.

Most enzyme reactions are inhibited below water activity of 0.85. Non-enzymatic browning reactions are dependent on water activity showing maximum around 0.6–0.7. Since water activity is a major factor influencing the keeping quality of a number of foods, it is obvious that packaging can play an important role to maintain optimal conditions for long storage life. Packaging aspects will be dealt with in another section.

4.4.3 Freezing of Water

During freezing of water, the water molecules arrange themselves in a tetrahedral fashion. These results in a hexagonal crystal lattice which is loosely built and has relatively large hallow spaces resulting in high specific volume. This is the reason for increase in volume of water on freezing. You must have observed ice cubes floating on water. The density of ice at 0° C is only 0.9168.

Water can exist in three phases, viz., solid, liquid and gas. The conditions under which they exist are separated by three equilibrium lines (Figure 4.4).

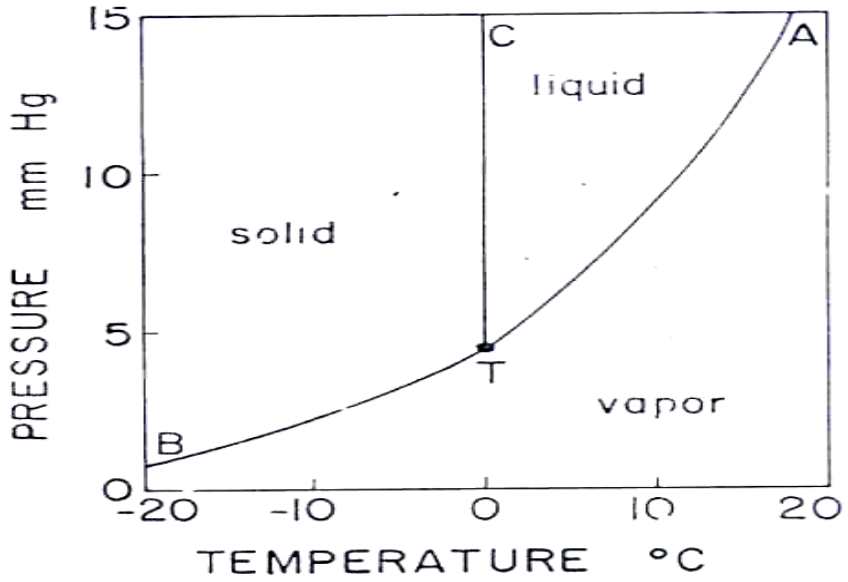

Figure 4.4: Phase diagram of water

The vapour pressure line TA, the melting pressure line TC and sublimation pressure line BT. They meet at the triple point T, where all three phases are in equilibrium. When ice is heated at pressures below 4.58 mm Hg it changes directly into vapour form (sublimation). This is the basis of freeze-drying.

Slow freezing will result in large ice crystal formation and rapid freezing in tiny ice crystal formation. Large ice crystals tend to damage the cell walls resulting in texture lose in frozen fruits, fish, meat, etc. During freezing of foods, water is transformed to ice with high degree of purity and solid concentration in the unfrozen liquid is gradually increased. This is accompanied by changes in pH, ionic strength, viscosity, osmotic pressure, vapour pressure and other properties. These changes along with the lower temperature are responsible for decrease in microbial activity and often on destruction of micro- organisms in frozen foods.

4.4.4 Water Quality and Standards

Water is used for different purposes in food processing. They include water used as ingredient in finished products, for generating steam, for cleaning raw materials, for cleaning plant and equipment, as heat exchange medium for heating and cooling etc. The water quality for different purposes varies.

In general, only potable water should be used in the preparation of food indented for human consumption. Potable water is that water which contains

no bacteria capable of causing human intestinal diseases and is aesthetically satisfactory for drinking purposes, i.e. free from undesirable odours and flavours.

Potable water should have good clarity, colourless and free from objectionable odour and taste.

Hardness of Water

Calcium and magnesium salts cause hardness of water. Permanent hardness is due to chlorides and sulphates of calcium and magnesium and temporary hardness is due to bicarbonates of these ions. Hardness is expressed as ppm (parts per million i.e. mg per litre) of $CaCO_3$ (calcium carbonate) on which basis water is classified according to degree of hardness (Table 4.4).

Table 4.4: Classification of water based on hardness

ppm of $CaCO_3$	Condition
Less than 50	Soft
50 to 100	Slightly hard
100 to 200	Hard
Above 200	Very hard

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 relationship between water activity and food spoilage.

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2. Why quick freezing better than slow freezing?

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3. Explain hardness of water.

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Microbiological Quality

The bacteriological quality of water used throughout the plant should meet the standards required for drinking water. The fitness of water for drinking purposes with respect to bacterial content is determined by the presence or absence of the coliform group of bacteria including Escherichia and Aerobacter species which indicate the possibility of faecal contamination. Waters drawn from deep wells or those purified by artificial means seldom show the presence of E.coli in 100 ml.

The quality of water required varies for different process food industries. The essential microbiological parameters of the BIS standards for water meant for general purposes are given in Table 4.5.

Table 4.5: Bacteriological tolerances

Sl. No.	Characteristic	Tolerance
1.	Coliform bacteria, MPN index/100 ml.	Less than 1
2.	Standard plate count, per ml., Max.	50 (Note 1)
3.	Proteolytic and lipolytic organisms, combined count per ml. Max.	5 (Note 2)

4.4.5 Chlorination

As in the case of municipal water supply, chlorination of industrial water has become a common practice in food processing plants as a means of improving plant sanitation. Gaseous chlorine or calcium and sodium hypochlorites are used for chlorination of water.

Chlorine Demand of Water

When chlorine is added to water other than distilled water, a small amount, normally 0.25 to 0.75 ppm, reacts with impurities in the water. This quantity of chlorine is called the **chlorine demand** of the water. The impurities responsible for chlorine demand include compounds containing iron, manganese, nitrites and sulphides. The chlorine, which reacts with these compounds, has no germicidal properties and cannot be measured by the methods used for testing chlorine concentration.

Break Point Chlorination

When chlorine is added to water, initially it is used up to satisfy the chlorine demand of the water. As additional chlorine is added, a free residual chlorine appears. At the same time, some chlorine loosely combines with nitrogenous matter present in water to form chloro-nitrogen compounds. The residual chlorine gradually increases until it reaches a concentration depending on the physical and chemical nature of the water, at which an oxidation reaction occurs between the free chlorine and the chloro-nitrogen compounds. The free residual nitrogen is decreased by the amount necessary to completely oxidise the chloro-nitrogen compounds. Further addition of chlorine beyond this point will result in a second rise in free chlorine concentration, which increases in almost direct proportion to the rate of chlorine application (Figure 4.5). The

point after the first rise in concentration at which the free residual chlorine reaches its lowest level is known as the break point. Break point chlorination is defined as chlorination to a degree where a persisting residual chlorine of 2-10 ppm occurs. The residual chlorine in water exists either as free chlorine or chlorine which has loosely combined with other elements. The rate at which bacteria exposed to chlorine are killed is proportional to the amount of chlorine present as hypochlorous acid.

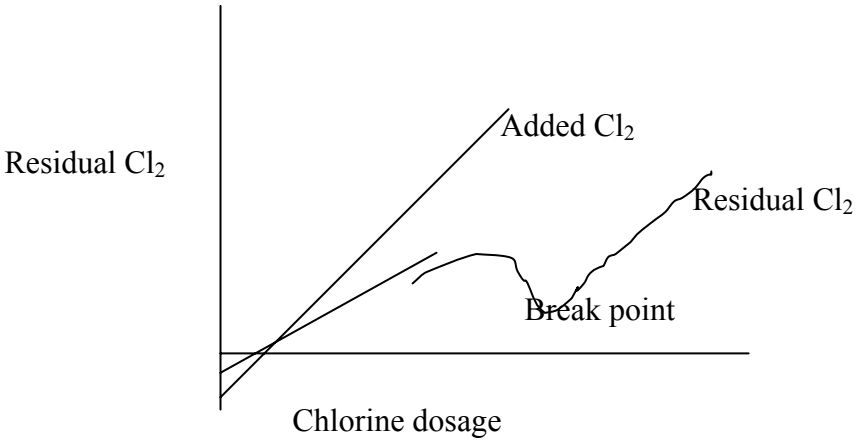

Figure 4.5: Break point chlorination of water

Chlorination of Water

In plant chlorination reduces bacterial count and clean up time and avoids odours. Chlorination of cooling water (for cooling canned products) prevents spoilage from recontamination. Residual chlorine of 5 ppm in the water is considered sufficient. Residual chlorine of 10-20 ppm is recommended for cleaning purposes. Chlorine concentration of 5 ppm has no effect on the flavour, odour or colour of canned products. For chlorination of water, hypochlorites are usually added in the form of stock solution containing 5000–10000 ppm of chlorine.

4.4.6 Packaged Drinking Water

As all of us know the quality of water supplied for drinking in many parts of our country is very poor and varies from place to place. Several diseases are spread through water. That is why today we find a booming packaged drinking water industry in the country. Water meant for producing packaged drinking water goes through a series of treatment and processes like filtration, reverse osmosis, ozonisation, etc., to obtain the required quality. PFA has laid down detailed specifications for packaged drinking water. The salient aspects of the specification are given in Table 4.6.

Table 4.6: Specifications for packaged drinking water

Sl. No.	Characteristics	Requirements
1.	Total soluble solids	Not more than 500 mg/litre
2.	PH	6.5–8.5
3.	Nitrates (as NO3)	Not more than 45 mg/litre
4.	Nitrites (as NO2)	Not more than 0.02 mg/litre
5.	Sulphide (as H2S)	Not more than 0.05 mg/litre
6.	Manganese (as Mn)	Not more than 0.1 mg/litre
7.	Copper (as Cu)	Not more than 0.05 mg/litre
8.	Zinc (as Zn)	Not more than 5.0 mg/litre
9.	Fluoride (as F)	Not more than 1.0 mg/litre
10.	Barium (as Ba)	Not more than 1.0 mg/litre
11.	Nickel (as Ni)	Not more than 0.02 mg/litre
12.	Chlorides (as Cl)	Not more than 200 mg/litre
13.	Sulphate (as SO4)	Not more than 200 mg/litre
14.	Calcium (as Ca)	Not more than 75 mg/litre
15.	Sodium (as Na)	Not more than 200 mg/litre
16.	Arsenic (as As)	Not more than 0.05 mg/litre
17.	Cadmium (as Cd)	Not more than 0.01 mg/litre
18.	Chromium (as Cr)	Not more than 0.05 mg/litre
19.	Mercury (as Hg)	Not more than 0.001 mg/litre
20.	Lead (as Pb)	Not more than 0.01 mg/litre
21.	Iron (as Fe)	Not more than 0.1 mg/litre
22.	Residual free chlorine	Not more than 0.2 mg/litre
23.	Yeast and mould counts	Absent in 250 ml
24.	E.Coli	Absent in 250 ml
25.	Coliform Bacteria	Absent in 250 ml
26.	Faecal streptococci and staphylococcus aureus	Absent in 250 ml
27.	Aerobic microbial count	Shall not exceed 100/ml.

4.4.7 Water Analysis

In the examination of water supplies, the test will depend on the purpose for which the water is used. The initial examination of water, or the testing of supplies from a new source may consist of the following:

I. Sanitary Examination

1. Physical characteristics:

   i) Colour, ii) Odour and taste, iii) Turbidity

2. Chemical characteristics:

   i) Total solids,
   ii) Organic matter,
   iii) Hardness,
   iv) Alkalinity,
   v) Acidity,

vi) pH,
vii) Nitrogen as nitrates, nitrites, free ammonia and albuminoid ammonia,
viii) Chlorides,
ix) Sulphates,
x) Free CO_2,
xi) Oxygen absorption, and
xii) Heavy metals.

II. Microbiological Examination

1. Plate count
2. Coliform count
3. Faecal streptococci test
4. Clostridium welchii test.

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. Which are the microorganisms or concern in potable water?

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2. Define chlorine demand and break point in chlorination.

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3. Which are the components of water analysis?

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4.5 LET US SUM UP

Proteins are macromolecules built up of amino acids. They form the building block of animal and human body structure. Pulses, oil seeds, and animal and marine foods are good sources of protein. The quality of protein greatly depends on its essential amino acid makeup.

Enzymes are biocatalysts, which catalyse biological reactions. All enzymes are proteins but all proteins are not enzymes. Several enzymes find application in food processing.

Water is an essential component of foods. Its state in foods plays an important role in food preservation. Quality of water used in food processing operations and for drinking is of paramount importance. Water is treated in several ways to make it suitable for different purposes.

4.6 KEY WORDS

Amino acids : Nitrogen containing compounds having both carboxyl and amino groups.

Essential amino acids : Amino acids which cannot be synthesised by the human body.

Albumin : Proteins, which are soluble in neutral salt free water.

Denaturation : Change in the structure of protein without breaking covalent bonds.

Immobilised enzymes : Enzymes fixed on water insoluble inert supports.

Chlorine demand : The quantity of chlorine added which reacts with the impurities in water and which does not show up as free chlorine.

4.7 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

Your answer should include the following points:

1. Aliphatic, aromatic, etc.
2. Different classes of simple proteins
3. Conjugated proteins

Check Your Progress Exercise 2

Your answer should include the following points:

1. Coagulation of egg albumin.
2. Deficiency of amino acids like lysine.
3. Pulses, oil seeds.
4. Valine, lysine, methionene, etc.

Check Your Progress Exercise 3

Your answer should include the following points:

1. Bio catalyst.
2. All enzymes are proteins.
3. Enzymes fixed on inert support.

Check Your Progress Exercise 4

Your answer should include the following points:

1. Relationship of water activity to microbial growth.
2. Large ice crystals damage cell structures.
3. Calcium and magnesium salts.

Check Your Progress Exercise 5

Your answer should include the following points:

1. Coliforms.
2. Reaction of added chlorine with impurities in water.
3. Depression in residual chlorine content.
4. Physical, chemical, microbiological.

4.8 SOME USEFUL BOOKS

1. Owen R. Fennema, (1976) Principles of food science, Part I- Food Chemistry, Marcel Decker Inc.; New York.

2. Meyer, L.H. (1969) Food Chemistry, Van Nostrand Reinhold Company, New York, Cincinnati, Toronto, London, Melbourne.

3. Braverman, J.B.S. (1963) Introduction to the Biochemistry of foods, Elsevier Publishing Company, Amsterdam, London, New York.

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

UNIT 5 FOOD CONSTITUENTS – VITAMINS AND MINERALS

Structure

5.0 OBJECTIVES

After reading this unit, you should be able to:

- explain the chemistry and properties of different vitamins;
- their physiological functions and deficiency diseases;
- their dietary sources; and
- describe the importance of minerals in human nutrition.

5.1 INTRODUCTION

You have already learnt about the macronutrients viz. carbohydrate, protein and fat. Besides these macronutrients, the human body requires certain accessory factors called vitamins for maintaining the health and well being. The accessory factors were named vitamins because of their vital importance to health. Some of the vitamins are unstable to the adverse storage conditions of foods and also many processing conditions. Since the vitamins are present in foods only in minute concentrations, their protection during preservation and processing of foods is a major concern.

The human body requires several minerals also for maintaining normal health. Iron and iodine deficiencies among populations are well known. Due to various reasons, the foods consumed by sections of the populations are deficient in vitamins and minerals. Therefore, these are some times added to foods. Since vitamins and minerals are collectively termed ‘micronutrients’, the process of adding them to foods is called micronutrient fortification. You will be learning these aspects in this unit.

5.2 VITAMINS

Vitamins are organic substances of very diverse composition required by the body usually in very minute quantities. Some of them, especially the B-group vitamins, take an active part in enzymatic reactions as co-enzymes. You will be learning more on enzymes in another unit. Most of the vitamins are supplied

to the body by plants. Vitamins do not supply energy to the body or any structural units for bodybuilding. They, however, play a most important role in the energy transfer as well as in control of many metabolic processes. Some of the vitamins like vitamin A occur in plant foods as provitamins (e.g. β-carotene), compounds that are not vitamins but can be transformed by the body into vitamins.

Vitamins are generally classified into two groups: a) fat-soluble vitamins i.e., vitamins soluble in fats and fat-soluble solvents (like petroleum ether, chloroform, carbon tetrachloride etc.) but not soluble in water, and b) water soluble vitamins, i.e., vitamins soluble in water but insoluble in fats or fat-soluble solvents.

5.2.1 Fat Soluble Vitamins

Fat soluble vitamins include 1) Vitamin A and Carotene (Provitamins A), 2) Vitamin D, 3) Vitamin E and 4) Vitamin K.

Vitamin A (Retinol)

Osborne and Mendel (1913) and McCollum and Davis (1917) showed that a fat-soluble factor present in butter was essential for the growth of rats on synthetic diet. The latter workers called the factor as "fat-soluble A". Moore (1930) showed that when large amounts of carotene are fed to vitamin A deficient rats, vitamin A was found in large amounts in liver, indicating the conversion of carotene to vitamin A in the animal body.

Chemistry and properties: In 1931, Karrer determined the structure of vitamin A. In 1937, Kuhn and Morris announced a method of synthesis if vitamin.

Vitamin A contains a β-ionone ring and a highly unsaturated side chain. Due to the unsaturated side chain, vitamin A is destroyed easily by oxidation. Being an alcohol, it forms esters such as acetate, succinate, palmitate etc. Vitamin A is stable to heat (100^{o}C) for short periods in the absence of oxygen. Vitamin A is slowly destroyed when exposed to light.

A large number of carotenoid pigments occur in nature. You will be learning their structure and properties in a later section in this unit. Carotenes are converted into vitamin A in the body. Among the carotenes, β-carotene has maximum vitamin A activity.

Functions and deficiency diseases/syndromes: Vitamin A has various functions in the human body. It is essential for the maintenance of normal vision, building and growth of skeletal cells and provides resistance power to the body. Dietary retinoids, especially carotenoid compounds have been found to suppress carcinogenesis (development of cancer).

The most important effect of deficiency of this vitamin in the diet is night blindness. In early stages of vitamin A deficiency, one cannot see well in dim light. In advanced deficiency, the subject cannot see objects in dim light. Night blindness is very common in regions where vitamin A intake is inadequate.

Dietary sources: Vitamin A is present only in fish liver oil and foods of animal origin such as liver, eggs, milk and fatty fish. Plant foods contain only carotenoids, the precursor of vitamin A. Among the plant foods, green leafy vegetables, carrot, mango and other yellow coloured fruits are good sources of carotenes.

Vitamin A levels are frequently expressed in International Units (I.U.). One I.U. equals 0.3 μg of crystalline vitamin A alcohol (retinol) or 0.6 μg β-carotene.

Vitamin D

Mellanby (1919) discovered that cod liver oil can cure or prevent experimentally produced rickets in dogs. McCollum and co-workers (1922) established experimentally that the antirachitic vitamin was different from vitamin A and called the factor vitamin D.

Chemistry and properties: Vitamin D is a group of compounds related to sterols. This vitamin occurs in several forms, the two most important are vitamin D_2 or ergocalciferol and vitamin D_3 or cholecalciferol.

Vitamin D is fairly heat stable. It is unstable on exposure to ultra-violet light. It is soluble in fat solvents.

Functions and deficiency diseases/syndromes: Vitamin D is essential for bone formation. It promotes absorption of calcium and phosphorus and deposition in bones.

In vitamin D deficiency, calcification (calcium deposition) of bone does not take place. This results in the disease called rickets in infants and children. The manifestations of the disease are: bowleg, enlargement of ankles and wrists and deformities of the chest bones called 'pigeon breast'. Exposure of children suffering from rickets to sunlight has a curative effect. Therefore, vitamin D is also called the sunshine vitamin.

Dietary sources: Vitamin D_2 occurs in small amounts in fish liver oils. Vitamin D_3 is widely distributed in eggs, milk, butter and cheese but large amounts occur only in fish liver oils.

One international unit (I.U.) of vitamin D is equivalent to the activity of 0.025 μg of pure crystalline vitamin D_2.

Vitamin E

Evans and Bishop (1922) discovered that for normal reproduction in rats, a fat-soluble factor present in crude vegetable oils was essential. They termed it vitamin E. Evans and co-workers (1936) isolated two compounds α and β-tocopherols possessing vitamin E activity.

Chemistry and properties: Tocopherols are derivatives of 6-hydroxy chroman with a phytyl side chain. Tocopherols are soluble in fat solvents and insoluble in water. Tocopherols have excellent antioxidant properties. The storage stability of unrefined vegetable oils is due to the tocopherols. They are slowly

destroyed in alkaline medium and the vitamin activity is destroyed by oxidation.

Functions and deficiency diseases/syndromes: Vitamin E is essential for normal reproduction in several species of animals and also in man. Its deficiency causes several disorders such as reproductive failure, liver necrosis (damage), muscular dystrophy, etc.

Dietary sources: Cereal germ oils like wheat germ oil and corn germ oils are the richest natural sources of the vitamin. Soybean oil is also a good source of tocopherols. Cottonseed oil is found to contain alpha, beta, and gamma tocopherols. Delta tocopherols were isolated from soybean oil. Since alpha tocopherols have the highest biological activity, its content is taken for calculating the human requirements.

One international unit (I.U.) of vitamin E is equal to the activity of 1 mg of synthetic α- tocopherols.

Vitamin K

As early as 1934, Dam and Schonheyder found that chicks fed on purified diets containing all vitamins known at that time developed haemorrhagic condition, which was cured by Lucerne (alfalfa) leaves. They named it vitamin K (Koagulations-vitamin) meaning vitamin responsible for blood coagulation.

Chemistry and properties: Vitamin K belongs to the group of compounds called quinones. Vitamin K_1 is called phylloquinone and K_2 is called menaquinone. Vitamin K_1 is 2-methyl 3-phytyl- 1,4-napthoquinone. It is the only vitamin K found in plants.

The commercially available vitaminK_1 is prepared synthetically from isophytol and a derivative of menadione. Vitamin K is also a fat-soluble vitamin.

Functions and deficiency diseases/syndromes: Vitamin K is essential for blood clotting by increasing the prothrombin levels in blood. Vitamin K deficiency leads to increased blood clotting time. This may lead to haemorrhage conditions. The deficiency can occur either due to inadequate intake or inadequate intestinal absorption of vitamin K. Inadequate intestinal absorption can occur due to disease of the liver or diarrhoea.

Dietary sources: Vitamin K occurs widely in plant foods, especially in leafy vegetables and also synthesised by the intestinal micro flora. It is assumed that almost 50% of the vitamin requirement is derived from intestinal micro organisms. Animal foods contain little vitamin K.

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 fat-soluble and water soluble vitamins? Name four fat-soluble vitamins.

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2. Which vitamin is required for normal vision? Why beta-carotene is called pro-vitamins A? List a few foods rich in beta-carotene.

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3. What is the chemical nature of vitamin K? List the physiological functions of the vitamin.

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4. Which vitamin is required for normal reproduction? List a few foods rich in the vitamin.

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5.2.2 Water Soluble Vitamins

Water soluble vitamins are classified into two groups viz.1) vitamins of the B group to which most of the water soluble vitamins belong and 2) vitamin C.

Vitamin B_1 (Thiamine)

Vitamin B_1 was the first of the B-vitamins discovered. A Dutch doctor, Eijkman, demonstrated in 1897 that a very common disease, beriberi, which prevailed at that time in the Dutch East Indians, was caused through feeding

only polished rice. Jansen and Donath (1926) isolated vitamin B_1 in crystalline form from rice polishings. This vitamin is also called thiamine.

Chemistry and properties: Williams and co-workers established the chemical structure of thiamine in 1936. Thiamine contains a pyrimidine ring and thiazole ring. It is a white crystalline powder. Sulphur dioxide destroys the vitamin activity of thiamine. Therefore, sulphites shoud not be added as a preservative to foods rich in thiamine. Thiamine is stable to heat in acid foods but it is less stable in neutral foods. It is destroyed by alkali. During wet processing, thiamine is leached out. Baking of cereal products shows considerable loss in thiamine content.

Functions and deficiency diseases/syndromes: Thiamine functions in carbohydrate metabolism. Free thiamine is readily absorbable by small intestine. It is necessary for nerve function, appetite and normal digestion.

Mild deficiency of the vitamin leads to loss of appetite, fatigue, depression and irritability. Severe deficiency causes a disease called Beriberi. There are three types of beriberi occurring in human beings viz. wet, dry and infantile beriberi.

Wet beriberi produces three general symptoms: a) Polyneuritis (inflammation of nerves), b) Oedema (swelling), and c) Disturbances of the heart. Muscle soreness and loss of reflex of the knee take place. The body tissues swell and oedema develops and it is noticed in the legs and thigh of the patient. Breathing becomes difficult and the heart becomes weak. Death occurs due to heart failure.

Dry beriberi affects the nerves of legs and arms. Calf muscles become tender and swollen. Toes and ankles get numb. The emaciated subject needs the help of sticks to stand and walk and finally becomes bedridden.

Infantile beriberi affects infants below 6 months. Two types of infentile beriberi are known. They are a) cardiovascular type, and 2) neuritic type.

Dietary sources: Whole cereals, pulses (legumes), oilseeds and nuts are good sources of thiamine.

Vitamin B_2 (Riboflavin)

In 1926, Goldberger and co-workers showed that pellagra was cured by autoclaved yeast, which was devoid of thiamine. This factor was called vitamon B_2. However, later studies showed that the vitamin so defined was a complex of several vitamins and riboflavin was one of them. Riboflavin does not cure pellagra.

Chemistry and properties: Riboflavin has a cyclic iso alloxazine nucleus and has a side chain containing a pentose sugar (ribose).

Riboflavin is slightly soluble in water and ethyl alcohol. The solution when exposed to ultra-violet light emits a strong greenish yellow fluorescence. Riboflavin is stable in acid or neutral medium but is destroyed in alkaline medium especially on heating.

Functions and deficiency diseases/syndromes: Riboflavin is concerned in the regulatory function of insulin. The retina contains riboflavin, which is converted by light to a compound involved in stimulation of the optic nerve. It forms a part of enzyme systems involved in the metabolism of carbohydrates, fats and proteins. Flavin Adenine Dinucleotide (FAD) and Flavin Mono Nucleotide (FMN) are formed from riboflavin.

Deficiency of riboflavin in the diet causes oral and facial, scrotal, vulval, and also ocular lesions.

Dietary sources: Liver, dried yeast, egg powder, whole and skim milk powder are excellent sources of riboflavin. Milk, cheese, eggs, whole grain and green leafy vegetables are also good sources.

Niacin (Nicotinic Acid)

The discovery of niacin was also associated with yeast extract as in the case of thiamine and riboflavin. It was isolated from liver and found that it can cure 'pellagra' in man and 'black tongue' in dogs. Niacin (nicotinic acid) is also called vitamin B_3.

Chemistry and properties: Acid or alkaline solutions of nicotinic acid on heating is converted to nicotinamide. It is one of the most stable of the vitamins. It is stable to acids, bases, oxidizing agents, heat and light. However, it is destroyed by autoclaving at 120^0C for 20 minutes. It is sparingly soluble in cold water, but soluble in hot water and alcohol. Nicotinamide exists almost exclusively as a constituent of coenzymes NAD (nicotinamide adenine dinucleotide and NADP (nicotinamide adenine dinucleotide phosphate).

Functions and deficiency diseases/syndromes: Nicotinic acid is essential for the normal functioning if the skin, intestinal tract and the nervous system. Deficiency of Niacin leads to a disease known as pellagra. In pellagra disease dermatitis, glossitis and stomatitis occur. Dermatitis is appears wherever that part of the body is exposed to sunlight. The other symptoms are irritability, mental anxiety and depression, which can develop to delirium and dementia.

Dietary sources: Yeast, liver, meat, poultry, wholegrains, fresh pork are excellent sources of niacin. Good proteins like milk protein are associated with niacin because triptophan, an amino acid, present in the proteins is converted into niacin in the body. It has been estimated that 60 mg of triptophan yield 1 mg of niacin.

Vitamin B_6 (Pyridoxin)

Pyridoxine is one of the vitamins of the B group, which prevents dermatitis. It was isolated in 1938 in pure form by different groups of workers. Pyridoxine is also called vitamin B_6.

Chemistry and properties: Pyridoxine does not belong to only one class of compound. It consists of three related substances namely pyridoxine, pyridoxal and pyridoxamine.

Pyridoxine contains a pyridine nucleus, two primary alcoholic groups and one phenolic hydroxyl group. Pyridoxal contains an aldehyde group in place of one primary alcoholic group and pyridoxal amine contains a primary amine side chain in place of a primary alcohol group. Pyridoxine is readily soluble in water and alcohol. It slowly gets destroyed when exposed to sunlight. Neutral or alkaline solutions of pyridoxine are heat labile. Oxidising substances like potassium permanganate and hydrogen peroxide also destroy it.

Functions and deficiency diseases/syndromes: Pyridoxine is essential for growth of infants. Its deficiency produces degeneration of the nerves. It has also some influence on the functioning of hormones. Besides pyridoxine play important roles in amino acid and lipid metabolism. Pyridoxal phosphate acts as a coenzyme for a number of enzyme systems. It removes carbon dioxide from the acid groups of certain amino acids and transfer amine groups from one compound to another. Pyridotal phosphate helps in transamination reactions, poryphyrin synthesis etc.

Dietary sources: Pyridoxine is widely distributed in both plant and animal foods. Dried yeast, rice polishing, wheat germ and liver are excellent sources. Whole cereals, legumes, oil seeds, nuts, egg, milk, meat and fish and leafy vegetables are good sources of this vitamin.

Pantothenic Acid

Pantothenic acid is one of the B group vitamins. In 1933, Williams reported that a factor present in yeast could prevent a specific type of pellagra (chick pellagra). This factor was named pantothenic acid.

Chemistry and properties: Pantothenic acid is an unstable, viscous oil. It is soluble in water. It contains an amino acid namely alanine. It is stable to heat but destroyed by acid and alkali. It is readily absorbed from small intestines.

Functions and deficiency diseases/syndromes: Pantothenic acid has an important role in the metabolism of Co-enzyme A. Therefore, indirectly pantothenic acid has a role in the utilization of carbohydrates and fats. Deficiency diseases of the vitamin are not often observed in man. However some of the deficiency disease symptoms are headache, fatigue, weakness, sleeplessness, nausea etc.

Dietary sources: It is widely distributed in foods. Dried yeasts, liver, rice polishing, wheat germs, fleshy foods, eggs, fish etc. are good sources of pantothenic acid.

Biotin

In 1916, Bateman showed that when rats were fed with uncooked egg white, they developed the peculiar skin disease. This condition was called egg-white injury. This was due to a toxic component called avidin. Gyorgi in 1931 isolated an anti-egg white injury factor from yeasts and named it as vitamin H. Later this factor was found in egg yolk and it was called Biotin.

Chemistry and properties: Biotin is sparingly soluble in cold water and freely soluble in hot water. It is stable to heat but sensitive to acid, alkali and oxidizing agents. It forms salts with alkali hydroxides.

Functions and deficiency diseases/syndromes: Biotin is essential for the activity of many enzyme systems. It helps in maintaining the skin structure and is necessary for normal gestation and lactation in animals. It is also required for fatty acid metabolism.

Biotin deficiency does not occur in humans frequently. Experimental deficiency in animals has shown skin scaling, dermatitis, muscle pains, anorexia (lack of appetite) and slight anaemia.

Dietary sources: Biotin occurs widely in foods of both vegetable and animal origin. Peanuts, chocolates, egg yolk, liver, kidney, peas, cauliflower, dry yeast, milk products, cereals etc. are good sources. Royal jelly from honeybee is the richest source of biotin (400 μg / 100 g).

Folic Acid

Wills (1934) showed that a vitamin present in autolysed yeast extract cured tropical macrocytic anaemia in humans. Mitchell, Snell and Williams (1941) reported the presence of this factor in spinach leaves essential for the growth of L-casei (a micro organism). They called this factor folic acid (*folium* meaning leaf).

Chemistry and properties: Folic acid is also called pteroyl glutamic acid. It is widely distributed in nature. It is a yellow crystalline compound moderately soluble in hot water and stable to heat. Many bacteria produce this vitamin.

Functions and deficiency diseases/syndromes: It is essential for the maturation of Red Blood Cells (RBC). It acts as co-enzyme in the transfer of single carbon groups such as methyl or formyl. It is essential for reproduction in animals. It also helps in the hair growth and health of skin.

Folic acid deficiency causes megaloblastic anaemia, which is also called macrocytic anaemia. This mainly occurs in pregnant women. This is due to accumulation of immature RBCs in bone marrow. Inadequate supply of folic acid causes glossitis (red sore tongue), diarrhoea, and anaemia.

Dietary sources: Dried yeast, green leafy vegetables, dry beans, cabbage, soybean, yeast, kidney and liver are good sources of this vitamin.

Vitamin B_{12}

In 1926, Minot and Murphy found that feeding liver in large quantities could control pernicious anaemia. In 1929, Castle also found that beef muscle is effective in controlling pernicious anaemia. Both these factors were responsible due to Vitamin B_{12}. It is also called Cyanocobalamin.

Chemistry and properties: Cyanocobalamin has a complicated chemical structure. It contains a porphyrin nucleus and a molecule of cobalt (4-5%).

Vitamin B_{12} is a water-soluble dark red crystalline compound not stable to acids and alkali. When it is exposed to sunlight it gets partially destroyed.

Functions and deficiency diseases/syndromes: Vitamin B_{12} promotes the maturation of Red Blood Cells (RBC). It is essential for the normal function of bone marrow and the nervous system. B_{12} takes part in many enzymatic reactions. It is essential for the absorption of Calcium and Phosphorus. It aids in providing energy to the central nervous system. It also helps in the increase of white blood cells and blood platelet count.

Deficiency of the vitamin causes pernicious anaemia. Life span of RBCs also comes down. The shape and size of RBCs also change. The other symptoms are skin lesions, reduction in gastric secretion, effect on spinal cord, tingling, numbness, loss of sense of limbs, depression etc.

Dietary sources: Vitamin B_{12} is present only in foods of animal origin. Kidney and liver, egg, cheese, milk, fish etc. are good sources of the vitamin.

Vitamin C (Ascorbic Acid)

Man knew the disease scurvy since centuries. It was found that sailors on long voyage were suffering from this disease. This was due to the non-availability of fresh fruits and vegetables to sailors in their long journey. Szent-Gyorgy (1928) isolated an acid with intense reducing properties from cabbage, orange and adrenal glands. Subsequently it was named ascorbic acid due to its antiscorbutic properties.

Chemistry and properties: Haworth and co-workers established the chemical structure of ascorbic acid in 1933. It is a six carbon mono basic acid present in its lactonised form. Its reducing property is due to the presence of a di-enolic configuration.Vitamin-C is a white water soluble crystalline compound stable in acid solution but sensitive to oxidation. Ascorbic acid has strong reducing property. It loses two hydrogen and forms dehydroascorbic acid. It is sensitive to high temperature. Vitamin-C is lost during food processing, storage and cooking.

Functions and deficiency diseases/syndromes: Vitamin C is essential for the formation of collagen and osteoblasts, for carbohydrate and cholesterol metabolism, for oxidation of phenylalanine to tyrosine, for the absorption of iron, and for rapid healing of wounds. Severe deficiency of the vitamin results in the development of the disease called scurvy. The disease is characterised by general weakness, spongy bleeding gums, loose tooth swollen joints and haemorrhages in various tissues.

Dietary sources: Ascorbic acid is widely distributed in the plant kingdom. Many fruits and vegetables like amla, orange, lemon, guava, cabbage, etc. are good sources of the vitamin.

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 beriberi?

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2. What is the chemical nature of niacin? Which disease is caused by its deficiency?

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3. What are the roles of vitamin B_{12} in the human system? List a few dietary sources of the vitamin.

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4. What is the chemical nature of ascorbic acid? Why it has reducing property? List a few foods rich in ascorbic acid.

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5.3 MINERALS

Proteins, carbohydrates and fats belong to organic compounds whereas minerals are inorganic in nature. When a food material is completely burnt, we are left with ash. This ash is nothing but minerals. These minerals are very essential to our body and they are included under the category called micronutrients.

The body contains about 24 minerals, all of which must be supplied by the diet. These minerals are necessary for the following different functions: 1) as

constituents of bones and teeth e.g. Calcium, phosphorus and magnesium; 2) as constituents of body cells of soft tissues like muscles, liver etc. e.g. Phosphorous; 3) as soluble salts which give to the body fluids and cell contents, their composition and stability which are both essential for life, e.g., sodium, potassium, chloride, and phosphorous; 4) some minerals are required in small quantities for specific functions, e.g., iron and copper for formation of hemoglobin; iodine for formation of thyroxine; zinc a constituent of a co-enzyme; cobalt the constituent of vitamin B_{12} and some other elements are essential for the activity of various enzymes. Minerals are classified into macro-minerals and micro minerals. Macro minerals are required in higher quantities and micro minerals are required in much smaller quantities. Calcium, phosphorous, magnesium, sodium and potassium are generally classified as macro minerals while iron, iodine, fluorine, manganese, cobalt, selenium, cobalt etc are classified under micro minerals. The physiological functions and dietary sources of some minerals are given in the following table.

Physiological functions of some minerals

Sl. No.	Mineral	Functions	Sources
1.	Calcium	Essential for the formation of bones and teeth. Absorption of Vitamin B12 and contraction of heart and muscles.	Milk and milk products, green leafy vegetables, ragi, egg and fish, etc.
2.	Phosphorus	Essential for formation and growth of bones and teeth, metabolism and transport of lipids and maintenance of acid base balance.	Rice and wheat bran, cheese, milk, meat and fruits and vegetables, etc.
3.	Sodium	Essential for acid base balance, regulation of osmotic pressure in cell fluids, maintenance of blood pressure and regulation of heart beat.	Table salt, milk meat, shell fish, egg, cheese, leafy vegetables, etc.
4.	Potassium	Essential for acid base balance, conduction of nerve impulses, conversion of glucose to glycogen and growth and build-up for tissues.	Fruits, dry fruits, milk, beans, etc.
5.	Magnesium	Takes part in the activity of more than 300 enzymes, vital for the functions of nerves, bones and muscles and teeth, role in coagulation of blood.	Green vegetables. Nuts, cereals, milk, etc.
6.	Iron	Component of haemoglobin, transport and storage of oxygen, essential for cell respiration.	Cereals, sea foods, meat, egg, vegetables, etc.

7.	Iodine	Normal thyroid function, deficiency causes goiter, i.e. Swelling of the thyroid gland and decrease of thyroidal hormone production.	Drinking water, Iodized salt, marine foods, meat, milk, fruits and vegetables, cereals, etc.
8.	Fluorine	Essential for bone and teeth formation, prevents tooth decay. Deficiency causes dental caries. Excess chlorine causes dental fluorosis.	Drinking water, milk, sea foods, garlic, tea, etc.
9.	Manganese	Takes part in the activity of many enzymes, helps in the development of bones, role in the regulation of fats and carbohydrates	Cereals, pulses , oil seeds, milk, vegetables, etc.
10.	Copper	Helps in the absorption and utilization of iron, influence on the oxygen supply, formation of melanin and phospholipids, takes part in enzyme activity.	Seeds, nuts, mushrooms, etc.
11.	Zinc	Aids in carbohydrate and protein metabolism, role in production of insulin, takes part in enzyme activity.	Cheese, fish, meat, egg, oyster, cereals, etc.
12.	Chromium	Influence on carbohydrate metabolism, stabilization of blood sugar level, influence on appetite.	Poultry, meat, milk, potato, nuts, etc.
13.	Selenium	Component of various enzymes, acts as an antioxidant, has protective effect against cancer.	Soybean, meat, fish, etc.
14.	Cobalt	Component of vitamin B12, Role in carbohydrate and lipid metabolism, role in synthesis of proteins.	Animal foods especially liver

Micronutrient Fortification

As compared to the major nutrients viz. carbohydrate, protein and fat, the other essential nutrients like the vitamins and minerals are required by the human system only in minute quantities. Therefore, they are termed as ‘micronutrients’. Due to lack of access to balanced foods, certain sections of the population suffers from severe micronutrient deficiency. To improve the quality of the diet, these nutrients are some times added to foods. This process is called ‘micronutrient fortification’. For example, iodine is added to table salt to prevent goiter and iron is added to wheat flour to prevent anemia. Vitamin fortified foods are also not rare. During fortification, the following precautions have to be taken.

1. Only the required concentration of the nutrient should be added to foods.
2. Fortification should not affect the stability of the food product.
3. It should not affect the colour and taste of the end product.

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. Which minerals are required for the formation of bones and teeth? List a few dietary sources for the minerals.

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2. Which diseases are caused by the deficiency of iodine and fluorine?

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3. Enumerate the importance of iron and cobalt in human nutrition.

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5.4 LET US SUM UP

Vitamins are complex organic substances required by the human body for several vital and well-defined physiological functions. They are mostly derived from plant sources. Because there is no structural relationship among the different vitamins, their classification is practically not possible. However, based on their solubility characteristics they are classified into fat soluble (soluble in fat and fat solvents) and water soluble vitamins. Most of the water-soluble vitamins belong to the group of the B complex. Many of the B complex vitamins take part in various enzymatic reactions as coenzymes.

The human body also requires several minerals for its physiological functions. Many of the minerals are cofactors of enzyme systems. Deficiency of some minerals cause specific diseases like for example, deficiency of iodine causes ‘goitre’ and deficiency of fluorine causes ‘dental caries’.

Since all the vitamins and minerals are not present in sufficient quantities in the staple diets of sections of the population, their deficiencies are common. In order to alleviate the deficiency, some foods are fortified with these nutrients.

5.5 KEY WORDS

Vitamin	**:**	Of vital importance
Retinol	**:**	Vitamin A alcohol
Provitamin A	**:**	Carotenes, which are converted to vitamin A in the body.
Liver necrosis	**:**	Liver damage due to disease.
Prothrombin	**:**	Substance in blood responsible for normal blood clotting.
Beriberi	**:**	The disease caused by the deficiency of vitamin B_1.
FAD	**:**	Flavin adenine dinucleotide.
FMN	**:**	Flavin mono nucleotide.
Pellagra	**:**	The disease caused by the deficiency of niacin.
Scurvy	**:**	The disease caused by deficiency of vitamin C.
Goitre	**:**	The disease caused by deficiency of iodine.
Dental caries	**:**	The disease caused by deficiency of fluorine.
Micro nutrients	**:**	Vitamins and minerals.

5.6 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   - Solubility in fat and fat-soluble solvents and water.
   - Vitamin A, D, E and K

2. Your answer should include the following points:

   - Vitamin A.
   - Beta carotene gets converted to vitamin A in the human body.
   - Green leafy vegetables, yellow fruits and vegetables like mango, papaya, carrot.

3. Your answer should include the following points:

    - Quinone
    - Blood clotting

4. Your answer should include the following points:

    - Vitamin E
    - Cereal germ oils, soybean oil, cottonseed oil

Check Your Progress Exercise 2

1. Your answer should include the following points:

    - Deficiency of vitamin B_1

2. Your answer should include the following points:

    - Nicotinic acid, nicotinamide
    - Pellagra

3. Your answer should include the following points:

    - RBC
    - Pernicious anaemia
    - Absorption of Ca, P
    - Animal foods eg. liver

4. Your answer should include the following points:

    - Six carbon monobasic acid
    - Di-enol
    - Citrus fruits, amla, guava

Check Your Progress Exercise 3

1. Your answer should include the following points:

    - Ca, P
    - Milk, ragi, leafy vegetables, rice and wheat bran, fish

2. Your answer should include the following points:

    - Goitre
    - Dental caries

3. Your answer should include the following points:

    - Haemoglobin
    - Anaemia
    - Oxygen transport
    - Vitamin B_{12}
    - Carbohydrate and lipid metabolism

5.7 SOME USEFUL BOOKS

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UNIT 6 FOOD ADDITIVES

Structure

6.0 OBJECTIVES

After reading this unit, you should be able to answer:

- the definition of food additives;
- different types of food additives;
- their chemical properties and functions in foods; and
- their legal status for the purpose of adding to foods.

6.1 INTRODUCTION

Food additives have been used for centuries to enhance the quality of food products. Smoke, alcohol, vinegar, and spices were used more than 10,000 years ago to preserve foods. Along with the developments in Food Chemistry and Food preservation in the early 1900s, the use of food additives increased significantly. The demand for new, tasty, convenient, and nutritious foods continued to increase. As a result, by the early 1960s, over 2500 different chemicals were being used in foods in many developed countries.

Different people used to understand food additive in different ways. The widely accepted definition of food additive is:

> "a substance or mixture of substances, other than a basic foodstuff, which is present in a food as a result of any aspect of production, processing, storage, or packaging. The term does not include chance contaminants".

Food additives are substances intentionally added for specific functions. The number of additives being used in food today is very large. However, they can be classified into a few types based on their functional properties. They may include: 1) preservatives, 2) antioxidants, 3) acidulants, neutralizers and buffers, 4) colouring agents, 5) flavouring agents, 6) sweeteners 7) nutritional additives, and 8) miscellaneous additives.

The use of food additives is well-accepted practice but not without controversy. There have been a number of concerns regarding the potential short-term and long-term risks of consuming these substances. Based on the available scientific information on the toxicological status of each additive and the likely quantity of that additive consumed through a particular food, the maximum permissible limits (Acceptable Daily Intake, ADI) of additives have been stipulated. As you will be learning in subsequent units, each country has laid down its own food legislation and The Prevention of Food Adulteration Act (PFA) and Rules, 1954, in India lists the additives permitted in different foods and their maximum permissible limits. You will also be learning that our food standards are in the process of harmonisation with international food standards. Therefore, it is likely that the present list of permitted additives under PFA may increase in the near future. Keeping these points in mind, in this unit some important and versatile food additives are included even though some of them may not be permitted under PFA at present. The nutritional additives viz. vitamins and minerals have already been dealt with in the previous unit; they will not be included in this unit.

6.2 PRESERVATIVES

From prehistoric times humans have attempted to preserve food products from the deteriorative effects of microorganisms. Some chemical food preservatives like salt, nitrites and sulphites have been in use for many years. Even though newer packaging techniques, processing and storage methods are able to preserve foods without chemical preservatives, even today these chemicals play a significant role in protecting the food supply mainly because preservation using chemical preservatives is cheaper and more convenient.

Under PFA, 1954, preservatives are classified into Class I and Class II preservatives. Class I preservatives are also called natural preservatives. They are, common salt, sugar, dextrose (glucose), spices, vinegar or acetic acid, honey, and vegetable oils. There is no restriction to the addition of Class I preservatives to any food.

Class II preservatives are, Benzoic acid and its salts, sulphurous acids and its salts, nitrates or nitrites, sorbic acid and its sodium, potassium and calcium salts, calcium or sodium propionates, lactic acid, sodium or calcium propionate, methyl or propyl parahydroxy benzoic acid, sodium diacetate and sodium potassium and calcium lactate.

Among the above preservatives, benzoates and sulphites are most widely used for preservation of fruit and vegetable products. Sorbates have been permitted for some products lately. Therefore, these and a few natural preservatives will be dealt with in this unit.

6.2.1 Class I Preservatives

1) *Salt (sodium chloride):* Salt has been used as a preservative since the beginning of recorded history. Pickling of fruits and vegetables and salting of fish and meat are widely practiced. The anti- microbial activity of sodium chloride is essentially related to its ability to reduce water activity (a_w) and create unfavourable conditions for microbial growth. As the water activity of the external medium is reduced, microbial cells are subjected to osmotic shock and rapidly lose water through plasmolysis. These results in the cells ceasing to grow and either die or remain dormant. Aside from the osmotic influence on microbial growth, other possible mechanisms include limiting oxygen solubility in the medium and toxicity of chloride ions.

   Sodium chloride-intolerant bacteria are inhibited by concentrations as low as 1%. Some bacteria like the lactic acid bacteria used in producing lactic fermented vegetables (you have learned in the units under 'Food fermentations') can tolerate from 6–15 % sodium chloride. In general, food borne pathogenic bacteria are inhibited by a water activity of 0.92 or less which is equivalent to sodium chloride concentration of 13%. That is why for salt curing, sodium chloride concentration of about 13% is commonly used.

   The inhibitory effect of sodium chloride is dependent on several factors particularly pH. As acidity increases, less sodium chloride is required to inhibit microbial growth.

2) *Acetic acid:* Synthetic vinegar (dilute acetic acid) and brewed vinegar are widely used as acidulants and antimicrobials. Vinegar pickles are common in our country.

   Acetic acid is more effective against yeasts and bacteria than moulds. Only acetic, lactic and butyric bacteria are markedly tolerant to acetic acid. As is the case with most other preservatives, acetic acid is also more effective at lower pH. Generally, 1-2 % acetic acid is sufficient to inhibit most of the organisms.

3) *Sugar and spices:* They also have preservative effects in many food products. The main function of sugar is to reduce the water activity of the medium thus inhibiting the growth of microorganisms. Many chemical substances in spices (terpenes) have been shown to have antimicrobial properties.

6.2.2 Class II Preservatives

As mentioned above, there are a number of chemicals having preservative action. However, only a few of them are permitted for use in foods. So, what are the factors to be considered in selecting a preservative for a food?

How to select a preservative?

Firstly the effectiveness of the preservative against different types of spoilage organisms must be known. This along with the knowledge of the common spoilage organisms associated with the product will allow the selection of the

correct preservative for the product. Secondly, the physico-chemical properties of both the preservative and the product should be known. The ionisation and solubility characteristics of the preservative in the product as well as the pH of the product are important factors. Finally, the safety and legality of the preservative chosen must be known.

Benzoic acid and benzoates: Benzoic acid is found naturally in cranberries, plums, prunes, cinnamon, cloves and most berries. It is a strong antimycotic agent. Most yeasts and moulds can be controlled using 0.05–0.1% benzoic acid. Control of many bacteria requires much higher concentration. Benzoic acid is sparingly soluble in water (0.27% at 18^{o}C). As sodium benzoate has higher solubility (66% at 15^{o}C), it is mostly used for preservation. Benzoates are most effective at low pH (pH 2.5–4.0) because the undissociated form is the effective antimicrobial agent.

Benzoic acid is permitted in several products like squashes, syrups, crushes, fruit juices, jams, jellies, marmalade, beverages, pickles and tomato products. You will be learning its maximum permissible limits in these products in the course on different products. Benzoic acid and its sodium and potassium salts have been generally recognised as safe (GRAS).

Sulphur dioxide and sulphites: Sulphur dioxide and its various salts have a long history of use dating back to the times of the ancient Greeks. They have been used extensively as antimicrobials and to prevent enzymatic and nonenzymatic browning in a variety of food products.

Sulphur dioxide is a colourless, non-flammable gas with a suffocating odour. It dissolves readily in water to produce sulphurous acid (H_2SO_3). Sulphur dioxide and its salts (bisulphites and metabisulphites) exist in a pH – dependent equilibrium in solution.

As the pH decreases, the proportion of the undissociated H_2SO_3 increases. As in the case of benzoic acid, the undissociated sulphurous acid has more antimicrobial activity than the dissociated ions.

Sulphurous acid inhibits yeasts, moulds and bacteria. However, yeasts and moulds are less sensitive than bacteria. That is the reason why sulphur dioxide is used at low concentrations (about 100 ppm) during grape juice fermentation to control the growth of other microorganisms and facilitate growth of yeast. Sulphur dioxide and sulphites are permitted under PFA for a number of products like fruit pulps, squashes, syrups, crushes, cordials, wines, RTS beverages, and dehydrated fruits and vegetables. Sulphur dioxide is also used as an antibrowning agent. Fruits are exposed to fumes of burning sulphur before drying to prevent browning and also insect and microbial attack. Sulphite solutions are also used as dip solution for vegetables before drying or dehydration.

Sulphur dioxide and several sulphites have GRAS status. However, sulphites cannot be used in meats and in foods that are sources of the vitamin thiamine. As sulphites have strong bleaching action on plant pigments like anthocyanins, they should not be used for preserving such products. It has been found that sulphites show allergic responses in certain individuals, such as steroid-

dependent asthmatics. This has led to ban of use of sulphites on raw fruits and vegetables in many countries.

Sorbic acid and sorbates: Sorbic acid and its sodium, potassium and calcium salts are collectively known as sorbates. Sorbic acid is present in some berries like berries of the mountain ash berry (rowanberry). It is a trans-trans, unsaturated monocarboxylic fatty acid.

The acid is a white crystalline powder and is slightly soluble in water (0.16% at 20^{o}C). The potassium salt, which is highly soluble in water (58.2% at 20^{o}C), is mostly used as the preservative. However for preservation of oils like corn oil, the acid is used because the salt is practically insoluble.

In the case of sorbic acid also, the undissociated acid has the highest antimicrobial activity. Therefore, sorbic acid is also more effective at low pH. However the dissociated acid also shows microbial action, though of a lower order. At pH above 6, the dissociated acid is responsible for more than 50% of the inhibition observed. This is the reason why sorbates are preferred for products like chapatti and cheese.

Sorbates inhibit most of the species of yeasts and moulds. Several species of bacteria are also inhibited by sorbates. At present under PFA, sorbates are permitted for only a few fruit and vegetable products. They include jams, jellies, marmalades, glazed or candied fruits, fruit bars, fruit juice concentrates and prunes. Some of the other products include cheese, flour confectionary, smoked fish, preserved chapatties and fat spreads. Sorbic acid and potassium sorbate have GRAS status.

Nitrites: Nitrites have been used in meat curing for many centuries. For meat curing, nitrite is used along with a mixture of salt, sugar, spices, and ascorbate. Nitrite contributes to the development of the characteristic colour, flavour, and texture improvement and preservative effects.

Nitrites are white or pale yellow hygroscopic crystals. Sodium nitrite is quite soluble in water. Nitrite has a strong inhibitory action against *Clostridium botulinum* and several other microorganisms. It is more effective below neutral pH (below 7.0). Along with salt, nitrite exhibits stronger antimicrobial action.

Biologically derived antimicrobials

Antimicrobial substances (antibiotics) produced by microorganisms have been known for many years. However, some of these substances are allowed for food use only in recent years. Nisin, and natamycin have been permitted in some foods.

Nisin is a polypeptide produced by *Steptrococcus lactis* (now called *lactococcus lactis*). The solubility of the compound depends on the pH of the medium. It is more soluble in acidic pH and almost insoluble in neutral pH.

Nisin has a narrow spectrum affecting only gram-positive bacteria, including lactic acid bacteria, streptococci, bacilli, and clostridia. It generally does not inhibit gram-negative bacteria, yeasts or moulds. The antimicrobial action of nisin is pH dependent, increases as the pH decreases. It is effective at very low

concentrations of the order of 0.04–2.0 ppm. Nisin has been permitted in packaged coconut water and canned rasagolla under PFA.

Natamycin: It is produced by the bacterium *Streptomyces natalensis*. The compound has a large lactone ring which is substituted with one or more sugar residues. Natamycin is primarily effective against yeast and moulds and is ineffective against bacteria, viruses and actinomyces. Natamycin is also effective at very low concentrations of the order of 5-10 ppm. Natamycin has been permitted for surface treatment of hard cheese.

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 additive.

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2. How are preservatives classified under PFA? List the Class I preservatives.

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3. List the permitted (PFA) Class II preservatives. What are the functions of sulphites in foods?

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6.3 ANTIOXIDANTS

Antioxidants play important role in preserving fats, oils and fatty foods. You have already learnt the chemistry of oils and fats. They are essentially fatty acid esters of glycerine. The fatty acids can be either saturated or unsaturated. The unsaturated fatty acids in fats and oils can undergo oxidation during storage leading to rancidity development. Along with rancidity development, vitamin destruction, discolouration and even toxic effects are possible.

Lipid oxidation is often referred to as an autocatalytic reaction. It is a complex reaction, which once initiated will become a chain reaction. Food antioxidants

are substances that are able to inhibit or interfere with the autoxidation reaction fundamental to glyceride oxidation. In simple terms, they get oxidised in preference to the fats and oils.

You have already learnt that tocopherols present in many vegetable oils have antioxidant property. Similarly, ascorbic acid and lecithin have antioxidant properties. However, the major antioxidants commercially used in foods, fats and oils are phenolic compounds and are generally referred to as phenolic antioxidants. Certain metals like iron and copper present in foods are strong catalysts of fat oxidation and may react with antioxidants to cause discolouration. Food acids like citric acid have the ability to bind these metals. Therefore, the antioxidants are usually added along with citric acid.

The most widely used phenolic antioxidants for fats and oils are i) butylated hydroxy anisole (BHA), ii) butylated hydroxy toluene (BHT), iii) propyl gallate and iv) tert-butyl hydroquinone (TBHQ).

Butylated hydroxy anisole (BHA): BHA is a white, waxy solid that is usually tableted to minimize caking during storage. It is readily soluble in glycerides and organic solvents and insoluble in water and has a distinct phenolic odour. BHA is quite stable during normal processing and storage of fatty foods. Therefore, it is considered to have good carry-through effect. However, being volatile at high temperatures, BHA may be lost partially during deep fat frying.

Butylated hydroxy toluene (BHT): BHT is a white, crystalline solid. It is also soluble in glycerides, and insoluble in water. It has a fair degree of carry-through effect but partially lost by volatilisation at high temperatures. BHT is less effective as an antioxidant than BHA.

Propyl gallate: Propyl gallate is the n-propyl ester of 3,4,5- trihydroxy benzoic acid or gallic acid. It is a white to light grey powder. It has low oil solubility and significant water solubility. Though it has very good antioxidative properties, due to its heat-labile nature; it has very little carry-through properties especially under alkaline conditions encountered in baked foods. Therefore, propyl gallate is used in combination with other antioxidants like BHA thus providing the combined effects of improved storage stability and carry-through protection.

tert-Butyl hydroquinone (TBHQ): TBHQ is a white to light tan crystalline solid noted for its effectiveness in increasing the storage stability of fats and oils. It is moderately soluble in fats and oils, and only slightly soluble in water. Unlike the other phenolic antioxidants, it does not form coloured compounds with metal ions in foods like iron, which is an advantage. TBHQ provides good carry-through protection to fried foods.

Applications of phenolic antioxidants

As mentioned earlier, among the food additives, perhaps the antioxidants are the most widely used. They are used in vegetable oils, meat products, confections and chewing gums, cereal products like breakfast cereals, bakery products etc. Use of the antioxidants in fruit and vegetable products though limited, is of considerable commercial importance. Some of them include fruit

nuts like walnut, almonds, cashew nuts; citrus oils, dehydrated potato products like powder, flakes and granules.

Under PFA, all the above phenolic antioxidants **except BHT** have been permitted with restrictions. Additionally, lecithin and ascorbil palmitate are also permitted for specific food products.

6.4 ACIDULANTS

Acidulants contribute a variety of functional properties that lead to the enhancement of food quality. Most of the acidulants used in food are organic acids. The organic acids and their salts commonly used in foods are acetic, ascorbic, citric, lactic, malic and tartaric acids. Inorganic acids like phosphoric acid is also used extensively in cola type beverages. Organic acids like citric, malic and tartaric acids are widely distributed in pants. Ascorbic acid, which is vitamin C, is also of plant origin. Lactic acid as the name implies is derived from milk. Since some aspects of acetic and ascorbic acids were already covered elsewhere, they are not specifically discussed here.

Citric acid is perhaps the most widely used organic acid. It is a tricarboxylic acid abundantly present in citrus and many other fruits. Even though it used to be produced from citrus fruits, at present most of the commercial citric acid is manufactured by fermentation. Citric acid is a white crystalline powder, easily soluble in water. Commercial citric acid is available as the monohydrate. It is hygroscopic in nature. Therefore, citric acid is not very suitable for use in dry food formulations.

Malic acid is 2-hydroxybutanoic acid, which is a dicarboxylic acid. It is the major acid in apples and mango. It is a crystalline white powder, easily soluble in water. Synthetic malic acid is available commercially. Since it is not hygroscopic, malic acid is preferred for use in dry food formulations.

Tartaric acid is also a dicarboxylic acid. It is the predominant acid in grapes and tamarind. It is also a white crystalline solid, soluble in water. It is usually extracted from the argol sediment formed during fermentation of grapes. Tartaric acid finds application in baking powder and effervescent ‘health salts’.

General functions of acidulants

The proper selection of an acid is dependent on which property or combination of properties of the acid is desired as well as its cost. Some of the general functions are given below.

- *Flavouring agents:* They intensify certain tastes and flavours, mask undesirable tastes
- *Buffering action:* The salts of organic acids, especially the sodium salts control the pH of food during various stages of processing as well as of the finished products.
- *Preservation:* By reducing pH, prevent growth of microorganisms and the germination of spores, which lead to spoilage and food poisoning.

- *Sequestering:* Bind metal ions and enhance the function of antioxidants.
- *Viscosity modifiers:* Like in dough, consequently modifying the shape and texture of baked foods.
- *Meat curing agent:* Together with other curing components, enhance colour, flavour and preservative action.

Most of the food acidulants are permitted under PFA with certain restrictions.

6.5 COLOURING AGENTS

Food colouring agents (colourants) may often be considered simply of cosmetic in nature, but the role they play is actually very important. You will be learning more about this quality attribute of food in a subsequent unit.

The addition of colourants to foods in order to make them more attractive is not a new invention. Extracts of spices and vegetables were used for the purpose as early as 1500 B.C. The advent of the use of food colourants in the late 1800s and early 1900s was unfortunately accompanied by their misuse in food adulteration, frequently to disguise food of poor quality. Some of these deceptive practices included colouring of pickles with copper sulphate; cheese with vermillion and red lead; tea with copper arsenite, lead chromate and indigo; and candy and turmeric with lead chromate, red and white lead and vermillion.

Development of synthetic dyes became a boon to the food industry because these colourants were superior to natural extracts in tinctorial strength (colour intensity), number of shades, stability, and easy availability and was cheap. However, safety is the most important aspect of synthetic food colours. Extensive toxicological studies have been carried out on these colourants in different countries. While many of the colourants have been found to be harmful, a few others appeared to be safe for use depending on various factors like the quantity of the colourant consumed. Besides, the test methods followed in different countries varied, which have resulted in much scientific and political debate. Consequently, colourants considered safe in one country may not be considered safe in another country. The toxicological studies are revealing newer information and hence the regulatory status of the colourants used in countries throughout the world is in a state of flux. One common observation is that the number of permitted synthetic colourants is decreasing year by year.

The regulatory status of several natural colourants is less severe mainly because they are mostly extracted from edible plant sources. Another class of colourants are called **nature-identical** colourants. They are those identical counterparts of naturally occurring pigments. Some examples of nature-identical colourants are β -carotene, canthaxanthine, and β -apo-8'-carotenal. Their regulatory status is similar to natural colourants.

Under PFA, the following natural and synthetic colours are permitted at present with restrictions on their maximum levels and the specific food products.

Natural colouring matters

1. Beta carotene
2. Beta – apo -8'-carotenal
3. Methyl ester of beta – apo - 8'- carotenoic acid
4. Ethyl ester of beta – apo - 8'- carotenoic acid
5. Canthaxanthin
6. Chlorophyll
7. Riboflavin (lactoflavin)
8. Caramel
9. Annato
10. Saffron
11. Curcumin (or turmeric)

Synthetic food colours

Sl.No.	Common Name	Shade	Chemical class
1.	Ponceau 4R	Red	Azo
2.	Carmoisine	Red	Azo
3.	Erythrosine	Red	Xanthene
4.	Tartrazine	Yellow	Pyrazolone
5.	Sunset yellow FCF	Yellow	Azo
6.	Indigo carmine	Blue	Indigoid
7.	Brilliant blue FCF	Blue	Triaryl methane
8.	Fast green FCF	Green	Triaryl methane

6.5.1 Natural Food Colourants

Anthocyanins: Anthocyanins are the intense red and blue water-soluble pigments occurring in many fruits, vegetables and flowers like strawberries, cranberries, raspberries, blueberries, grapes (blue), Jamun, and some flowers. Anthocyanins are composed of an aglicone (anthocyanidin) esterified to one or more sugars and may be acylated. The sugars may be glucose, rhamnose, galctose, xylose and arbinose. Grape skin and elderberries are good commercial sources of anthocyanin pigments.

The anthocyanin double ring benzopyran structure is very reactive. The compounds are easily ionised and tend to become colourless above pH 4.5. They exibit their most intense colours below pH 3.5. Therefore, these colourants are only suited for acidic foods. Anthocyanins easily undergo discolouration in the presence of amino acids, phenolic sugar derivatives etc. They are also bleached by ascorbic acid and sulphites.

Carotenoids: Carotenoids are responsible for the yellow, orange and red pigments in a number of plants and animal foods. Carotenoids are classified into three groups. i) Carotenes – These are hydrocarbons containing β-ionone rings and possess vitamin-A activity. Ex. β-Carotene present in carrots, chillies, soybean. ii) Lycopenes – These are carotenoids not having β-ionone rings and do not possess vitamin-A activity. Lycopene is present in tomato, apricot, watermelon, and red guavas. iii) Xantohphylls – These are oxygenated derivatives of carotene. These have β-ionone rings, but do not possess vitamin-

A activity. They are present in papaya, orange peel, and yellow maize. iv) α-Carotene – This is similar to β-carotene in its biological activity.

Chemically carotenoids are poly –enes composed of isoprene units. They are fat soluble and fairly heat stable. During processing of fruits and vegetables, partial loss of carotene takes place. They are stable at pH 2–7. As a result of their chemical structure, which contains conjugated double bonds, carotenoids are very sensitive to oxidation. Ascorbic acid can protect β -carotene by serving as an antioxidant. "Nature-identical" synthetic β -carotene is marketed in forms that confer protection from oxidation.

Betalains: Betalains are found in plants such as red beets, amaranthus flowers, bougainvillea, cactus fruits etc. Betalain colours range from red to yellow. The red beet is the most common commercial source of these pigments. Betalains are sensitive to pH, light and heat. These compounds are most stable at pH 4 – 5. Because of the carbohydrates present in betalains, the colourants tend to impart beet flavour to the food.

Production of colourants like anthocyanins and betalains by tissue culture technique offers the advantage of a more reliable supply of the colours independent of plant variability and elimination of the strong undesirable plant flavours.

Chlorophylls: Chlorophylls, the most abundant naturally occurring plant pigments, are the green and olive green pigments in green plants. Chlorophylls are obtained from a wide variety of sources and they are mixture of 2 compounds namely chlorophyll a and chlorophyll b present in the ratio of 3a: 1b in plants. They belong to a group of important biological pigments called porphyrins, which include haemoglobin, and is composed of four pyrole rings held together. Magnesium is located in the centre of the molecule.

Chlorophylls are soluble in alcohol, diethyl ether, benzene, acetone etc. but insoluble in water. Some metal ions like iron, zinc and copper react with chlorophyll and the green colour becomes brighter. In alkaline pH, the colour of chlorophyll is better retained. Chlorophylls are heat sensitive and during processing of fruits and vegetables containing chlorophyll, the green colour is lost and turns brown. When vegetables containing chlorophyll is cooked, the central Mg atom is replaced by hydrogen atom and loses it's colour forming pheophytin. Chlorophylls may be stabilized by replacement of the magnesium ion in the compound with copper.

Curcumin is the main colourant (yellow) in the oleoresin obtained from turmeric (*Curcuma longa*). Curcumin is fat-soluble, has good tinctoriasl strength, but exhibit slight sensitivity to light, air and pH.

Paprika oleoresin, which is orange red to deep red, is the extract of mild capsicum (*Capcicum annum*). Like curcumin, paprica oleoresin is also water insoluble. Paprika and turmeric oleoresins are available in various standardized forms.

Saffron is generally stable toward light, oxidation and pH and has a high tinctorial strength.

6.5.2 Synthetic Colourants

Synthetic colourants, also known as coal tar dyes or aniline dyes were earlier manufactured from coal tar derivatives. Although the colourants were highly purified before they were added to foods, the negative connotation of their association with coal tar resulted in much unfavourable publicity. As a result, synthetic colourants are no longer manufactured from coal tar derivatives but instead are developed from highly purified petrochemicals.

As can be seen from the table above, the permitted synthetic colorants belong to five chemical classes viz. azo, xanthene, pyrazole, indigoid and triarylmethane. You may notice that all together there are three red, two yellow, two blue and one green colour permitted under PFA. All these colours are water-soluble. As mentioned earlier, these colours are more resistant to chemical reaction, pH and heat compared to natural colourants.

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 the function of an antioxidant in foods?

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2. List two natural and three phenolic antioxidants.

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3. Describe the general functions of acidulants in foods?

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4. List the synthetic colours permitted in foods under PFA.

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6.6 FLAVOURING AGENTS

Flavour like colour of foods has a great bearing on acceptance of foods and therefore, has enormous commercial importance. It apparently has no nutritional value although some studies have indicated that taste can alter intestinal absorption of glucose and fat metabolism. Flavour is defined in several ways. One definition is: "**sensation produced by a material taken in the mouth, perceived principally by the senses of taste and smell, and also by general pain, tactile and temperature receptors in the mouth".**

Food flavours is a very vast subject. You will be learning sensory perception and analysis of flavour in a subsequent unit. In this section you will learn some essential aspects of the chemistry and application of food flavourings.

During the early days, people used spices to enhance or modify the flavour of foods. Along with the developments in synthetic chemistry and analytical techniques like gas chromatography and mass spectrometry, there was a spurt in synthetic flavour compounds and identification of the flavour compounds in various foods and processed products. The role of sensory analytical techniques in flavour research is very significant. One major finding was that most of the food flavours were due to a combination of a number of chemical compounds and only in a few cases, one single compound was responsible for the characteristic flavour of a food. Few examples are menthol in peppermint, benzaldehyde in bitter almond, citral in lime peel, amyl acetate in ripe banana, cinnamaldehyde in cinnamon etc. Therefore, it became apparent that creation of a food flavour is not an easy task. However, with a combination of art and scientific know-how, today a large number of flavour formulations are available. It is an extremely large industry. Food flavourings are classified into three groups.

1. *Natural flavours and natural flavouring substances:* They are flavour preparations and single substances respectively acceptable for human consumption, obtained exclusively by physical means from vegetable, sometimes animal raw materials, either in their natural state or processed, for human consumption.

2. *Nature-identical flavouring substances:* They are substances chemically isolated from aromatic raw materials or obtained synthetically, they are chemically identical to substances present in natural products intented for human consumption, either processed or not.

3. *Artificial flavouring substances:* They are those substances, which have not been identified, in natural products intended for human consumption either processed or not.

The natural flavours include spice oleoresins and oils, essential oils like citrus oils; fruit aroma concentrates like apple aroma concentrate etc. As indicated earlier, the number of synthetic flavour substances is extremely large. Therefore, instead of listing the permitted flavouring substances, only those, which are not permitted, are specified under PFA. In this connection it is important to note that the concentrations of the flavour chemicals in natural or synthetic flavours to impart the desired flavour perception are extremely low,

of the order of parts per million or parts per billion. As they have self-limiting property, maximum permissible limits are not stipulated.

6.7 SWEETENERS

Sweetness is one of the important taste sensations. The importance of sweetness is reflected in huge production of sugar (sucrose) world over. Sucrose is not consumed only for its sweetness. It also has many functional properties in foods like a bulking agent, texture modifier, and preservative.

Like any other carbohydrate, sucrose is also a nutrient providing energy to the human system. Over the years, sucrose has been implicated in obesity development and associated diseases and also dental caries. Besides, diabetes has become a common disease among large sections of the population. As a result there is a general trend towards reducing energy intake. This has resulted in development of sucrose alternatives.

There are two types of sucrose alternatives viz. nutritive and non-nutritive sweeteners. Nutritive sweeteners also called calorie sweeteners are usually carbohydrates or carbohydrate derivatives. Non-nutritive sweeteners include a range of natural products and some synthetic chemicals.

6.7.1 Nutritive Sweeteners

You have already learnt about glucose, glucose syrup, fructose and high fructose syrup and their relative sweetness in an earlier unit. In this section you will be learning about a few other nutritive sweeteners.

Sorbitol: Sorbitol is a six carbon sugar alcohol that was originally found in the berries of mountain ash. It is chemically produced from glucose for commercial use. It is highly soluble in water (72% at 25^{o}C). Sorbitol has half the sweetness of sucrose.

As it has a much lower caloric content compared to sucrose, sorbitol is used as a sweetener for diabetic foods, sugar-free candies and chewing gums.

Xylitol: Xylitol (xylit) is a pentiol found in most fruits and berries as well as xylan (a polysaccharide) containing plant materials. It is also produced by microbiological methods. Xylitol is a crystalline substance, having good water solubility (64 % at 25^{o}C). It has sweetness and caloric content equal to sucrose. However, because xylitol is absorbed slowly, it does not cause increase in blood glucose level as glucose or sucrose. Therefore, it is used in diabetic foods also.

Isomalt: Isomalt is also called hydrogenated isomaltulose. It is an equimolar mixture of 6-0-β-D-glucopyranosyl-Dglusitol and 1-0-β-D-glucopyranosyl-D-mannitol. Isomalt is produced by the enzymatic transglucosidation of sucrose to isomaltulose and hydrogenation. It is of about half the sweetness of sucrose. It is stable in acid and alkaline media under conditions normally occurring in food processing. It has no impact on blood sugar. Isomalt is used as a sugar substitute in confectionaries, chewing gum, soft drinks and desserts.

6.7.2 Non-Nutritive Sweeteners

Saccharin: Saccharin was synthesised way back in 1879. During the two world wars, the use of saccharin as a sweetener increased due to the scarcity of sugar and became an accepted sweetener for special dietary and dietetic foods even though its safety has repeatedly been questioned.

Saccharin is a general name used for saccharin, sodium saccharin and calcium saccharin. Chemically saccharin is 1,2-benzisothiazol-3 (2H)-one-1, 1-dioxide. Saccharin and sodium saccharin are white crystalline powders soluble in water. They are about 500 times sweeter than sucrose. It has good stability during cooking and baking of food products but leaves a slight bitter metallic aftertaste. It is permitted as a sweetener in several countries including India with restrictions.

Available toxicological information does not conclusively implicate saccharin for any serious health hazard. The Acceptable Daily Intake (ADI by WHO) of saccharin is fixed at 2.5 mg/Kg body weight. However, as more and more research results accumulate, safer alternates for saccharin is bound to emerge.

Cyclamates: Although sodium cyclamate was synthesized in 1937, its actual use as a sweetener started only in 1950. Cyclamates is a group name used for cyclamic acid, sodium cyclamate and calcium cyclamate. They are synthesized from cyclohexylamine by sulphonation. They are not found in nature. Cyclamates are stable at high temperatures, are easily soluble in water. They are about 30 times sweeter than sucrose and can be used as a noncalorie sweetener in a variety of products. Some times it is used as a mixture along with saccharin.

Cyclamates are not without safety questions. Therefore its usage is only allowed with restrictions like most other non-nutritive sweeteners. The use of cyclamates is not permitted under PFA. Its ADI value is 11 mg/Kg body weight.

Aspartame: Aspartame was discovered only in 1960. It is the methyl ester of L-aspartyl-L-phenylalanine. Aspartame is produced from the amino acids phenylalanine and aspartic acid. It is an odourless white crystalline powder, slightly soluble in water and almost 150-200 times sweeter than sucrose.

Aspartame provides 4 Kcal/g energy. Aspsrtame provides sugar like sweetness in foods, but under certain moisture, temperature and pH conditions, it is hydrolysed and loses its sweetness. Therefore, aspartame is more suitable for dry products or as a table top sweetener although it is widely used in soft drinks, dairy products etc. Soft drinks are usually sweetened with a mixture of saccharin and aspartame.

Available evidence suggests that normal consumption of aspartame is safe because consumption of asprtame from foods is far below any suspected toxic levels. Its ADI value is fixed at 40-mg/Kg body weight. The use of asprtame is permitted in many countries including India.

Acesulfame K: Acesulfame K is one of the most recently introduced (1967) non-nutritive sweeteners. Acesulfame K is the potassium salt of 6-methyl-

1,2,3-oxathizine-4(3)-one-2,2-dioxide. It is a white crystalline powder, freely soluble in water, non hygroscopic and 150-200 times sweeter than sucrose. Acesulfame K is used in soft drinks, chewing gum and as a table-top sweetener. More food applications are being investigated.

The available toxicological data on acesulfame K do not show any serious safety implications. The ADI value is fixed at 9-mg/Kg body weight. Its use as a sweetener is permitted in some countries including India with restrictions.

6.8 MISCELLANEOUS ADDITIVES

The number of substances in this category though very large, is not used extensively in fruit and vegetable products. Therefore, only a few of them will be dealt with in this section. They include emulsifiers and stabilizers, firming agents, anticaking agents, clarifying agents etc.

Emulsifying and stabilizing agents are essentially used for emulsifying and stabilizing dispersions of oils and fats in aqueous media. They include different types of gums (you have learnt under 'carbohydrates'), esters of fatty acids, lecithin, ester gums (glycerol esters of wood rosin) etc.

Firming agents like calcium chloride are used to firm the texture of canned fruits and aluminium sulphate added to pickles.

Anticaking agents are used to impart free flowing properties to dry products. Examples are silicates in potato flakes, dehydrated vegetable powders, cocoa powders, salt; tricalcium phosphate in spices, and fruit powders; and starches in icing sugar etc.

Clarifying agents are used to clarify fruit juices and wines and chill proofing of beer. Gelatin is a typical example of a clarifying agent.

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. Differentiate between natural and nature-identical flavouring substances.

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2. What is the difference between nutritive and non-nutritive sweeteners? Give three examples of each.

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3. Give one example each for Firming agent, Anticaking agent and Clarifying agent.

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6.9 LET US SUM UP

A large number of additives are used in foods for different functions. Some are used as preservatives, which prevent microbial spoilage while antioxidants preserve food against oxidative deterioration. Acidulants have dual roles of preservation as well as imparting the desired taste. Colourants and flavouring agents are added essentially to enhance the acceptability of foods. There are also other additives having functions like texture modification, clarification, imparting free flowing characteristics to food powders etc.

All the food additives are not free from suspected health hazards. Therefore, their use in foods is restricted by food legislations.

6.10 KEY WORDS

Additive : Substance added intentionally

Class I preservatives : Natural preservatives

Class II preservatives : Chemical preservatives

Antioxidant : Substance, which prevents oxidation

Nutritive sweeteners : Sweeteners having calorific value.

6.11 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   - Other than basic foodstuff
   - Added, not chance contaminant

2. Your answer should include the following points:

   - Class I and class II preservatives
   - Salt, sugar, spices, vinegar etc.

3. Your answer should include the following points:

    - Benzoates, sulphites, sorbates etc
    - Antimicrobial, anti browning

Check Your Progress Exercise 2

1. Your answer should include the following points:

    - Inhibit autoxidation of glycerides
    - Get oxidised in preference to fats and oils

2. Your answer should include the following points:

    - Tocopherols, ascorbic acids
    - BHA, BHT, TBHQ

3. Your answer should include the following points:

    - PH reduction
    - Buffering
    - Flavouring

4. Your answer should include the following points:

    - Ponceau
    - Carmoisine
    - Erythrosine
    - Sunset yellow etc

Check Your Progress Exercise 3

1. Your answer should include the following points:

    - Natural flavouring substances isolated by physical means
    - Chemically identical to natural substances but chemically isolated from aromatic substances or synthetically prepared

2. Your answer should include the following points:

    - Calorie sweeteners
    - Sucrose, glucose, fructose etc
    - Non calorie sweeteners
    - Synthetic
    - Saccharin, cyclamate, aspartame, acesulfame K

3. Your answer should include the following points:

    - Calcium chloride
    - Tri calcium phosphate
    - Gelatine

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UNIT 7 ETHYLENE LIBERATION AND ITS CONTROL

Structure

7.0 OBJECTIVES

After going through this unit, you should be able to:

- know the role of ethylene in fruit physiology;
- tell how ethylene is synthesized in fruit tissues;
- explain the mechanism of action of ethylene; and
- state the different methods to remove ethylene from storage rooms.

7.1 INTRODUCTION

Ethylene is the simplest chemical compound known to cause a significant physiological effect. It is also known as ripening hormone and is effective at very low concentration. Ripening is the result of complex changes; many of them probably occur independent of each other. Respiration and ethylene production are the two of the major processes occurring during ripening. Ethylene plays a role in post-harvest life of many horticultural crops, which is often deleterious, speeding senescence and reducing shelf life, but sometimes beneficial, improving the quality of the product by promoting faster, more uniform ripening.

Ethylene is a gaseous harmone with a characteristic suffocating sweetish odour. It is both an anaesthetic and asphyxiant. High vapour concentration can cause rapid loss of consciousness and perhaps death by asphyxiation. Removal to fresh air usually results in prompt recovery if the person is still breathing. When the gas is handled in liquefied form, skin and eye burns can result from contact with the liquid. Cases in which liquid ethylene contacts, a physician must see to the eye.

7.2 SOURCES OF ETHYLENE

Ethylene gas is relatively inexpensive industrial chemical, but it is often more convenient or safer to provide ethylene by means other than the gas. Regardless of the source of ethylene, the treatment conditions are important for the ripening process.

Explosion-proof Ethylene Mixtures

Using mixtures of ethylene with inert gases can eliminate the danger of explosions from oversupply of ethylene to a ripening room. The proportion of the inert gas should be such that at high concentrations of ethylene not enough oxygen remains in the ripening space to provide an explosive mixture. Ripegas, a commercial formulation of ethylene contains 6 percent C_2H_4 in CO_2 by weight.

Ethylene Generators

Ethylene generators, in which a liquid products ethylene when heated in the presence of a catalyst, are now widely used for supplying ethylene in ripening rooms. The liquid comprises of ethanol and agents that catalyze its dehydration.

Ethephon

Ethephon (2-chloroethyl phosphonic acid) is strongly acidic in water. In solutions above pH of about 5, the ethephon molecule spontaneously hydrolyses, liberating ethylene molecule. Ethephon is commercially available (Ethrel, Florel, Cepa) and is used for preharvest treatment on a variety of crops for controlling developmental processes, or inducing ripening. For enhancing postharvest ripening, it has the disadvantage as it has to be applied to the fruit in liquid form as a spray or as a dip. This extra step in handling may cause microbial infection. But compared to ethylene treatment, it has the advantage, as no special facilities are required to ripen fruit with ethephon, if the ambient temperatures are within the range required to ripen the commodity.

Calcium Carbide

Heating calcium oxide with charcoal, under reducing conditions, readily produces calcium carbide. When hydrolyzed, calcium carbide produces acetylene, containing trace amounts of ethylene that are sufficient to trigger fruit ripening. Simple generators can be used in partially vented spaces to ripen or degreen fruits. In some instances CaC_2 wrapped in newspaper can be used as the generator. Water vapour from the fruit releases sufficient ethylene from CaC_2 to cause ripening.

7.3 USES OF ETHYLENE

Post-harvest Uses

Post harvest uses of ethylene include its uses as ethephon, an ethylene-releasing chemical, as a growth regulator. Fruit ripening is by far the largest

application of ethylene gas in post-harvest technology. Other uses of ethylene in crops include-

Flower and Sprout Induction

The stimulation of flowering of pineapples by ethylene treatment is important for pineapple industry. Japanese bulb growers discovered that iris bulbs from fields that had been burned at the end of the season to control leaf diseases flowered earlier and more prolifically than controls. It was found that smoke did the same for bulbs that had been harvested, and smoking of bulbs is still practiced in Japan. The active ingredient in the smoke is ethylene, and it has now been shown that ethylene treatment of the propagules of a number of flowering crops stimulates flowering. Narcissus bulbs normally do not flower without ethylene treatment, and treatment with ethylene for a few hours just after lifting induces almost 100 per cent flowering.

Ethylene is also used as a short treatment to enhance the sprouting of seed potatoes. Ethylene treatment breaks the dormancy of the buds, but prolonged treatment inhibits their extension growth.

Shuck Loosening and Fruit Release

Ethylene is also used to induce the abscission of leaves, flowers, and fruit from many plants. Pre-harvest application of ethylene to walnut and pecan trees induces shuck loosening and improves harvest efficiency. Similarly, these chemicals are used to loosen the abscission zone on the stalk of fruits that are mechanically harvested (e.g. sour cherries), for improving harvest yields.

Chlorophyll Destruction

In many plant tissues, ethylene treatment result in rapid loss of cholorphyll, the green colour in leaves and unripe fruit. This property is used for degreening of citrus, where the orange colour is revealed as the chlorophyll is destroyed during ethylene treatment. Ethylene also helps in bleaching of celery and accelerated curing of tobacco.

Fruit Ripening

The concentrations of ethylene (in the range of 0.1 to 1 ppm) are required for the ripening in most of climacteric fruits. The time of exposure to initiate ripening depends on type of fruit, but usually exposures of 12 hours or more are usually sufficient. Full ripening sometimes takes several days after the ethylene treatment. The effectiveness of ethylene in achieving faster and more uniform ripening depends on the type of fruit being treated, its maturity, the temperature and relative humidity of the ripening room, ethylene concentration and duration of exposure to ethylene.

Control of temperature is critical to good ripening with ethylene. Optimum ripening temperatures are 18° to 25°C. At lower temperatures ripening is slowed; at temperatures over 25°C bacterial growth and rotting may be accelerated, and above 30°C ripening may be inhibited. Fruit that have been cool-stored must be warmed to 20°C to ensure that ripening proceeds rapidly. As ripening starts, the burst of respiration can generates heat, therefore it is

essential to ensure that this heat does not increase pulp temperatures to the point where ripening is inhibited

7.4 ETHYLENE AS RIPENING INDUCER

Ethylene is regarded as an agent that can induce ripening. It has been established that all the fruits produce minute quantity of ethylene during development, but climacteric fruits produce much larger amounts of ethylene as compared to non-climacteric fruits during ripening. Further, the internal ethylene concentration of climacteric fruits varies widely, but that of non-climacteric fruits changes little during development and ripening. A concentration of 0.1-1.0 µl/l ethylene for one day is sufficient to hasten ripening of climacteric fruits, but the magnitude of climacteric is relatively independent of the concentration of ethylene. In contrast, applied ethylene merely increases the respiration of non-climacteric fruits, and the magnitude of increase depends upon the concentration of ethylene. Further, the rise in respiration in response to ethylene may occur more than once in non-climacteric fruits in contrast to single respiration increase in climacteric fruits.

The significance of ethylene for fruit ripening was established during the early part of this century when heaters burning kerosene were used to degreen or colour yellow lemons. Ethylene was regarded as an external agent that could promote the ripening of fruit, and other plant tissues, produced extremely small quantities of ethylene.

7.5 BIOGENESIS OF ETHYLENE

It was shown that application of an amino acid methionine greatly stimulated ethylene production in apples, and this compound was then considered to be the starting point for ethylene biosynthesis. Then s-adenosyl-methionine (SAM) was identified as another important compound in pathway, which is converted to 1-aminocyclopropane-1-carboxylic acid (ACC). ACC is now regarded as the immediate precursor for ethylene and ACC synthase controls the rate at which pathway operates. ACC synthase is activated by an enzyme cofactor, known as pyridoxal phosphate. Inhibitors of enzyme that require pyridoxal phosphate, such as aminoethoxy vinyl Glycine (AVG) and amino-oxyacetic acid (AOA) can be used to inhibit ethylene production.

It is generally agreed that the amino acid methionine is the precursor of ethylene in plants, with the conversion having an absolute requirement for molecular oxygen. Small amounts of ethylene probably can also be formed in plant tissues from the oxidation of lipids involving a free radical mechanism. As yet, the ethylene-producing system has not been isolated from fruit tissues for in vitro studies and the site or the organelle where ethylene is synthesized is still not very clear. The unravelling of the bio-chemical pathway of ethylene biosynthesis in plants has been one of the most interesting bio-chemical stories of recent years. Application of the amino acid, methionine greatly stimulated ethylene production in apples, and this compound was then considered to be the starting point for ethylene biosynthesis. Researchers at Davis, USA identified SAM (S-adenosyl-methionine) as another key compound in the pathway and then, almost simultaneously, Amrhein in West Germany and Adams and yang at Davis, USA discovered that SAM was converted to an

unusual cyclic amino acid, ACC (1 aminocyclopropane-1-Carboxylic acid) which is now thought to be the immediate precursor for ethylene.

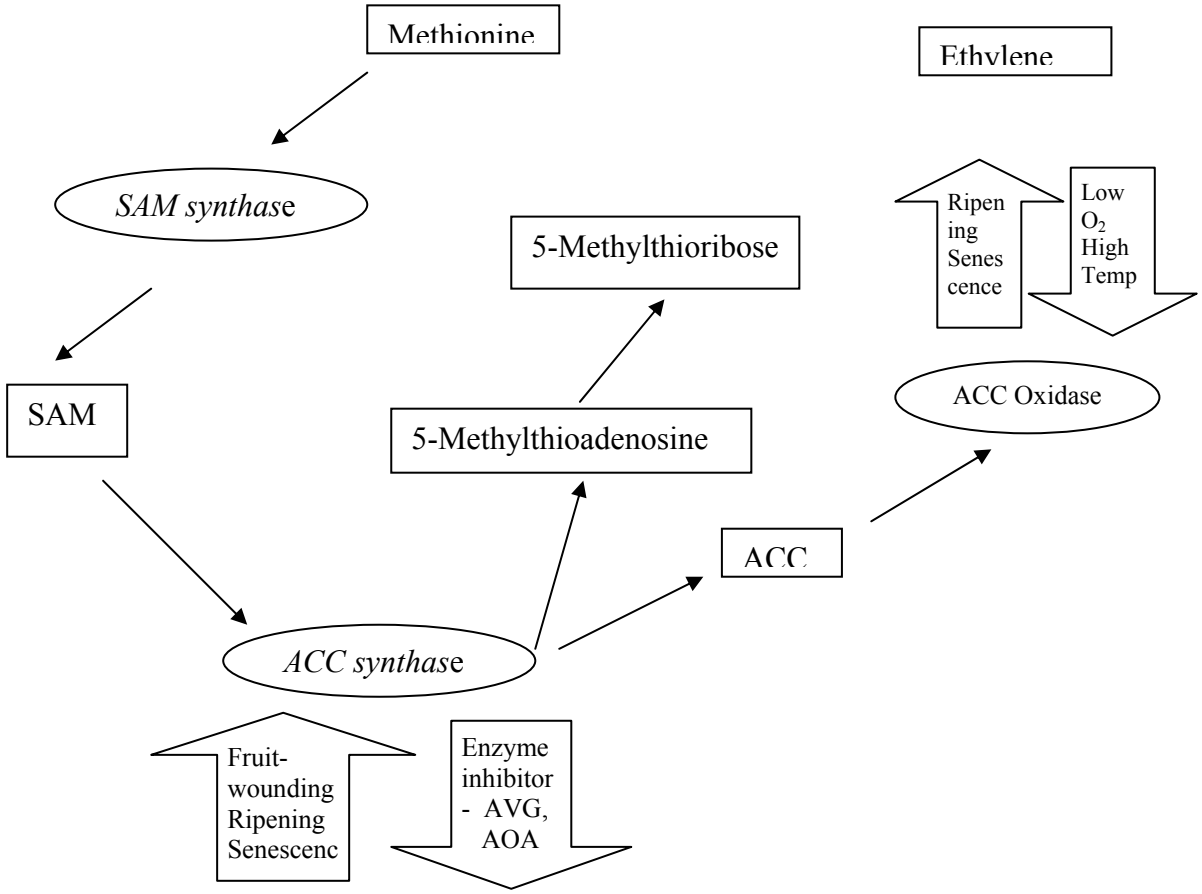

Figure 7.1: Biosynthetic pathway of ethylene

The enzyme which controls the rate at which the pathway operates, ACC synthase, is activated by a common enzyme co-factor, pyridoxoal phosphate such as AVG (amino-ethoxyvinyl gylcine) and AOA (aminooxyacetic acid) which inhibit ethylene production. Cobalt ion and low O_2, which inhibit the final step in the pathway, the Acc oxidase can also reduce ethylene production.

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. Name the precursor of ethylene biosynthesis.

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2. How ethylene effect differs in climacteric and non-climacteric fruits?

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3. What is the role of ethylene in post-harvest life of horticultural produce?

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4. Name the factors regulating action of ACC synthase enzyme?

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7.6 MECHANISM OF ETHYLENE ACTION

Although applied ethylene will initiate the ripening of climacteric fruits and will cause some ripening like changes in non-climacteric fruits similar to those in senescing tissue, it has not been possible to prove that ethylene acts in vivo as a true plant hormone, that is, acts in fruit tissues at trace levels, in promoting and controlling fruit ripening. A considerable amount of circumstantial evidence is available which suggests that ethylene, probably in concert with the other plant hormones (auxins, gibberellins, kinins and abscisic acid) exercises hormonal type control over the fruit ripening process. The relationship of the other plant hormones to ripening is as yet not clearly defined.

In some fruits, such as banana, avocado and melons, there is a small rise in endogenous (internally produced) ethylene concentration preceding the commencement of the respiratory climacteric. For example, the internal ethylene concentration of Honey Dew melon rises from the pre-climacteric level of 0.04 microlitre/litre to 3.0 microlitres/litre at which concentration the

fruit commences to ripen. Other fruits, such as mango and apple, do not show this rise in internal ethylene concentration before ripening. Once ripening has commenced, the large amount of ethylene synthesized by climacteric fruits is thought necessary to promote all of the aspects of ripening.

It is well known that many fruits as they develop and mature, become more sensitive to ethylene. For sometime after anthesis (flowering) young fruit can have high rates of ethylene production. Early in the life of fruit can have high rates of ethylene production. Early in the life of fruit the concentration of applied ethylene required to initiate ripening is high and the length of time to ripen is prolonged, but decreases as the fruit matures. The tomato is an extreme case of tolerance to ethylene. Banana and melons, in contrast, can be readily ripened with ethylene even when immature. Nothing is known about the factors that control the sensitivity of the tissue to ethylene.

There is also no clear evidence to suggest the mechanism by which ethylene initiates and controls fruit ripening and little is known about the site of action of ethylene and the mechanism by which ethylene either promotes ripening or increases respiration in non-climacteric fruits or in other tissues such as the potato tuber. Ripening has long been considered to be a process of senescence and to be due to a breaking down of the cellular integrity of the tissue; some ultrastructural and bio-chemical evidence supports this view. There is now considerable evidence for ripening being a phase in the differentiation of plant tissue, with altered nucleic acid and protein synthesis occurring at the commencement of the respiratory climacteric. Both views fit in with the known degradative and synthetic capacities of fruit during ripening, but this type of study is unlikely to determine how ethylene initiates ripening.

Although applied ethylene initiates ripening in fruits, it is not proved how ethylene acts in vivo as a true plant hormone. Some evidence suggests that probably in combination with other hormones it exercises hormonal type control over the fruit ripening process. Some fruits show a rise in internal ethylene concentration before ripening, whereas others do not. The factors that control the sensitivity of the tissues to ethylene are yet to be known. But once ripening has commenced the large amount of ethylene produced by climacteric fruits is thought to be necessary to promote all aspects of ripening. It has been proposed that two systems exist for the regulation of ethylene biosynthesis. System I is initiated or perhaps controlled by an unknown factor, probably involved in the regulation of senescence. System I then triggers system II, which is responsible for production of large amounts of ethylene in climacteric fruits. Non-climacteric fruits lack an active system II.

Recently a model for the way in which ethylene induces a host of effects was introduced. According to this ethylene binds to a protein, called a binding site, and stimulates the release of a so called second message instructing the DNA to form mRNA molecule specific for the effects of ethylene. These molecules are translated into protein by polyribosomes, and the proteins so formed are the enzymes that cause the actual ethylene response.

7.7 ETHYLENE TREATMENT SYSTEMS

For ethylene treatment ripening rooms or specially built chambers with automatic control of temperature, humidity, and ventilation are used. It is not

essential that the rooms be hermetically sealed, but they should be as tight as practicable to prevent leakage. Because of the rapid increase in respiratory heat production following ethylene treatment, ripening rooms should be equipped with refrigeration systems adequate to hold the temperature.

Several methods are used to provide the proper ethylene concentration in the ripening room.

The "shot" System

In the shot system, measured quantities of ethylene are introduced into the room at regular intervals. The shots may be applied by weight, or by flow. The required ethylene application is made by adjusting the regulator to give an appropriate flow rate and, the time of delivery of the gas. Any piping leading into the ripening room should be grounded to prevent possible electrostatic ignition of the explosive concentrations of ethylene that are always present near the orifice when ethylene is being introduced. Sometimes the ethylene is administered by weight also.

Because the room containing the product being ripened is sealed in the shot system, CO_2 accumulates in the room and may inhibit the ripening process. Therefore the room should be well ventilated before each application, particularly if it is well sealed, by opening the doors for about half an hour. In large ripening rooms, ventilating fans should be provided. Where the ripening rooms are near rooms used for storage or handling of ethylene-sensitive commodities, the room should be ventilated to the exterior to prevent contamination.

The Trickle or Flow-through System

The ethylene is introduced into room continuously, rather than intermittently. As the flow of ethylene is very small, it has to be regulated carefully. This is usually done by reducing the pressure using a two-stage regulator and passing the gas into the room through a metering valve and flowmeter.

To prevent a build up in either CO_2 or C_2H_4 fresh air is drawn into the ripening room at a sufficient rate to ensure a change of air every 6 hours. The air is vented through a small exhaust port to the rear of the room.

A convenient way of monitoring gas being supplied in a trickle system is a simple "sight glass" in which, the ethylene bubbles through a water trap on its way to the ripening room. As in the shot system, correct temperature maintenance and adequate air circulation are essential for good ripening.

7.8 CONTROL

Ethylene is produced whenever organic materials are stressed, oxidized or combusted. There are many sources of ethylene pollution during post-harvest handling of perishables, but the most important are internal combustion engines, ripening rooms and ripening fruits. Other sources are aircraft exhaust, decomposing produce and sometimes fungi growing on it, cigarette smoke, rubber materials exposed to UV light and virus infected plants. Sometimes ethylene contamination of flowers takes place from propane-powered floor

polishers. The undesirable effects of ethylene that must be controlled are as follows:

Accelerated Senescence

In green tissues, ethylene commonly stimulates senescence, as indicated by loss of chlorophyll, loss of protein, and susceptibility to desiccation and decay. Ethylene pollution can result in yellowing of leafy vegetables (spinach, fresh herbs, parsley, broccoli and other green vegetables). The senescence of some flowers is also stimulated by ethylene at very low concentrations. These effects occur in flowers where increased ethylene production is part of natural senescence (sweet peas) and in others where it is not a part of natural senescence (roses).

Accelerated Ripening

Although acceleration of ripening is a beneficial use of ethylene, it can also be undesirable, as the presence of ethylene in the cucumber causes premature yellowing. Most climacteric fruits senescence faster if ethylene is present in the atmosphere, therefore ethylene in the storeroom may reduce storage life. The firmness of kiwi fruit in storage was dramatically reduced at 20 ppb ethylene concentration in the cool store.

Induction of Leaf Disorders

In many plants, exposure to ethylene results in darkening or death of portions of their leaves. This response is commonly seen in foliage plants and is of major economic consequence in lettuce, where ethylene causes the disorder known as russet spotting. In lettuce, the browning results from collapse and death of areas of cells following increased synthesis of phenolic compounds in response to ethylene.

Sprouting

The ethylene-stimulated sprouting is useful in propagules, but undesirable in commodities intended for consumption. Sprouting of potatoes increases water loss, leading to early shriveling and makes them unmarketable.

Abscission of Leaves, Flowers and Fruits

Ethylene induced abscission is most often a problem in ornamental plants, where low concentrations can cause complete loss of flowers or leaves. The Christmas cactus is sold when the first flowers are open, but exposure to ethylene may cause all the flowers fall in the bottom of the box during transportation.

Toughening of Asparagus

Ethylene stimulates the lignification of xylem and fiber elements in the growing asparagus spear, leading to undesirable touchiness and reducing the portion of the spear that is edible.

Induction of Physiological Disorders

Ethylene sometimes induces or hastens the appearance of physiological disorders of stored commodities. Rapid ripening of apples with low calcium contents induces high levels of the bitter pit storage disorder. Similarly, high ethylene levels in the storage chamber reduce the effectiveness of controlled atmospheres in maintaining quality of apples. While useful in inducing flowering in bulbs and other propagules, ethylene damages these propagules after the flowers have started to develop.

A number of techniques have been developed to protect sensitive commodities from the effects of ethylene. Selection of the appropriate method obviously depends on the commodity and the handling techniques used in its marketing.

Removing ethylene from the atmosphere around the commodity is the preferable method of preventing deterioration of ethylene sensitive products. Most of the times, removing the sources of ethylene can do it. Combustion gases exhaust should be avoided from handling and storage rooms. A good sanitation by removing overripe and rotting produce will help in reducing ethylene levels. Ventilation of storage rooms can also help in reducing ethylene concentration.

Eliminating Sources of Ethylene

High levels of ethylene in storage and handling areas can be avoided by removing sources of ethylene. In particular, commodities sensitive to ethylene, should be handled using electric forklifts. Internal combustion engine vehicles should be isolated from handling and storage areas and engines should never be left idling in an enclosed space during loading and unloading operations. Where these techniques are not feasible, it is possible to fit combustion engine exhausts with catalytic converters, which will reduce C_2H_4, emissions by 90 percent. Rigorous attention to sanitation will remove overripe and rotting produce which can be a source of ethylene.

Ventilation

Where the air outside storage and handling areas is not polluted, simple ventilation of these areas can reduce ethylene concentrations. An exchange rate of one air change per hour can readily be provided by installing an intake fan and a passive exhaust.

Chemical Removal

Ethylene can be removed by a number of chemical processes; the most important are described below:

- Using potassium permanganate
- Using ultraviolet lamp (by ozone)
- Activated or brominated charcoal
- Catalytic oxidizers

Potassium permanganate: Commercial materials, such as Purafil, utilize the ability of potassium permanganate ($KMnO_4$) to oxidize ethylene to CO_2 and

H_2O. The requirements for such materials are a high surface area coated with the permanganate and ready permeability to gases. Many porous materials have been used to manufacture permanganate absorbers, including vermiculite, pumice and brick. The type of material may depend on the purpose for which the absorber is required. For removing ethylene from room air, the absorber should be spread out in shallow trays, or air should be drawn through the absorber system.

Ultraviolet lamps: Commercial equipment for ultraviolet lamps draws the air from the storage room through the lamps. Ultraviolet lamps produce ozone, which is an active ethylene-removing agent. As the ozone produced by the lamps is very toxic to fresh produce, it must be removed from the produce.

Activated or brominated charcoal: Charcoal air purifiers, especially if brominated, can absorb ethylene from air. These systems are largely confined to use in the laboratory, as potassium permanganate absorbers are cheaper and more widely available.

Catalytic oxidizers: If ethylene and oxygen are combined at high temperature in the presence of a catalyst (e.g. platinized asbestos) the ethylene will be oxidized. Ethylene scrubbers overcome the difficulty of heating the incoming air by using the bed of ceramic as a heat sink and reversible flow of gas through the bed. These scrubbers are very efficient in reducing the ethylene concentration in the air to 1/100th of original concentration.

Bacterial systems: Approximately 30,000 metric tons of ethylene is liberated into the atmosphere each day from internal combustion engines, but the concentration of ethylene in air remains very low. The bacteria that use ethylene as a bio-chemical substrate are able to remove ethylene from the atmosphere.

Hypobaric Storage

Hypobaric storage helps in reducing the levels of ethylene as the relative concentration of all the gases in storage room goes down. Many of the benefits of hypobaric storage are due to reduction in the partial pressure of oxygen, which accompanies reduction in the atmospheric pressure.

Inhibition of Effects of Ethylene

Sometimes it is not possible to ensure low concentration of ethylene. In such cases attempts should be made to inhibit the effect of ethylene. Different techniques used are:

- Controlled atmosphere storage
- Use of antiethylene compounds (NBD, STS)
- Inhibition of ethylene biosynthesis

Controlled atmospheres: Low concentrations of O_2 and high concentrations of CO_2 in the storage atmosphere reduce the rates of respiration, ethylene production and other metabolic processes. CO_2-enriched atmospheres also may inhibit the action of ethylene on tissues sensitive to it. Accumulation of CO_2 produced by the fruits may help in preventing the action of ethylene.

Use of anti-ethylene compounds: Compounds that inhibit the action of ethylene include silver ion and 2, 5 norborneadiene (NBD). Complex between silver and thiosulfate (STS) is being used for ornamental commodities. It has a very low stability constant and therefore it moves readily from the vase solution to the head of cut flowers. Flowers pulsed with this material last two to three times as long as control flowers. Potted flowering plants do not lose their flowers during transportation if they are first sprayed with STS.

Inhibition of Ethylene Biosynthesis

Ethylene may reduce quality even when it is not present as a pollutant if the tissue itself produces ethylene. Inhibitors of ethylene biosynthesis, such as AVG and AOA have been used in laboratory experiments to extend flower vase life and fruit storage life but not on commercial scale.

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. Write the mechanism of ethylene action?

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2. How hypobaric storage helps in controlling ethylene?

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3. Name the chemical processes that can remove ethylene from storage environment.

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4. How one can minimize the deleterious effects of ethylene?

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7.9 LET US SUM UP

Ethylene, a ripening hormone plays an important role in post-harvest life of many commodities. Sometimes its effect are beneficial where it is used for uniform ripening or degreening, but mostly it is considered deleterious as it hastens senescence and reduces post-harvest life. Simple practices of good sanitation and ventilation of storage room can help in reducing ethylene concentration. Use of chemicals or advanced storage techniques can also reduce the deleterious effects of ethylene.

7.10 KEY WORDS

Physiology : Study of the functions and vital processes of living organisms or their parts.

Ethylene : A colourless flammable gas which stimulates ripening.

Ripening : The advance stage in the development at which fruit and vegetable are suitable for consumption/ utilization.

Climacteric : Fruits/vegetables showing a sudden upsurge in respiration couples with ethylene evolution.

Non-climacteric : Fruits/vegetables who do not show a sudden upsurge in respiration couples with ethylene evolution.

Biosynthesis : The formation of chemical compounds by the enzyme action of living tissues.

Hormone : A substance which is synthesized at one tissue and has specific effect on another tissue.

Ventilation : To circulate fresh air or drive out foul air.

Hypobaric : Less than normal air pressure.

Degreening : Removal of chlorophyII pigment form the tissue.

7.11 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answers should include following points:

   - Amino acid
   - Methionine

2. Your answers should include following points:

   - Ethylene concentration
   - Increase in respiration

3. Your answers should include following points:

   - Senescence
   - Decolourization

4. Your answers should include following points:

   - Wounding
   - Ripening
   - Enzyme inhibitors

Check Your Progress Exercise 2

1. Your answers should include following points:

   - Binding site
   - Polyribosomes

2. Your answers should include following points:

   - Relative concentration
   - Oxygen level

3. Your answers should include following points:

   - Potassium permanganate
   - UV rays
   - Charcoal

4. Your answers should include following points:

   - Controlled atmosphere
   - Antiethylene compounds

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UNIT 8 GROWTH, MATURATION AND SENESCENCE

Structure

8.0 OBJECTIVES

After going through this unit, you should be able to:

- know the chemical changes taking place during growth, ripening and senescence;
- tell about respiratory climacteric;
- explain the factors affecting respiration; and
- mention the morphological changes in fruit tissues during ripening and senescence.

8.1 INTRODUCTION

The life of fruits and vegetables can be divided into three major physiological stages following germination- growth, maturation and senescence. Ripening is considered to begin during the later stages of maturation and to be the first stage of senescence. Development and maturation of fruit are completed only when it is attached to the plant, but ripening and senescence may proceed on or off the plant. Major changes in carbohydrates, organic acids, pigments and volatiles are observed during growth of the produce, which continue during ripening and senescence.

Food may be stored in various storage organs, such as roots, shoots tubers, rhyzomes, bulbs, corms, fruits and seeds. Storage may take place at different seasons of the year and may in some plants be controlled by the length of the day, the length of the night period and the day & night temperatures. In many plants that live for more than one season (perennials), accumulation in the underground storage organs takes place at a rapid rate in the fall of the year.

Fresh fruits, as well as fresh vegetables are essential components of human diet. Both contain a number of nutritionally important compounds, such as vitamins, which cannot be synthesized by the human body; vitamin C is the

most important and essential nutritive substance found mainly in fruits and vegetables.

The fruits are used as a table commodity whereas the vegetables are usually cooked and then served as food. Some of the vegetables are "fruit-vegetables" and most of the vegetables are the other vegetative organs of the plant that include root, stem, flower, shoot, leaves and associated parts.

Fruits and vegetables are highly perishable products with active metabolism during the post-harvest period. Proper handling plays an important role in increasing their availability. On removal from the parent plant, vegetative parts, such as fruits, roots, stems etc are deprived of their normal supply of minerals, water, and also in some instances, simple organic molecules [e.g. sugars, hormones] that normally would be translocated from other parts of the plant. Innumerable physiological and biochemical processes are initiated and continued in the edible plant tissues at the time of harvest. Although the photosynthetic activity is negligible, most tissues remain capable of transforming many of the constituents already present in them. The diversity of metabolic shifts, which are specific to a given commodity [and often variety] are manifest in events such as 'rotting', 'ripening', 'sprouting', 'scald', 'brown core', 'hard core', 'toughening', and 'yellowing'.

The kind and intensity of physiological activity in detached plants determines their storage longevity. Some plant parts, such as seeds, fleshy roots, tubers, bulbs are morphologically and physiologically adapted to maintain the tissue in a dormant state until environmental conditions becomes favourable for germination or growth. Metabolic activity, though depressed, is not completely halted in such tissues. Fleshy fruits are unusual in that maturation is followed by a ripening process, which is associated with the development of optimal eating quality.

The diversified visible physiological changes, like sprouting, browning, toughening etc are desirable in some commodities and undesirable in others in relation to the eating quality. Almost all such changes are observed during ripening.

Physiology of Ripening

The term "ripening" is generally referred to the physical and biochemical changes taking place in the fruits after the cessation of growth till the onset of senescence and decay. The ripening process is dependent upon maturity, since a given stage of development must be attained before ripening progresses. The process of ripening continues while the fruit is on the tree, but the damage caused by the birds, insects etc makes it uneconomical to allow it to ripen on the tree. Hence, the fruits are usually harvested at the horticulturally mature stage. Fruits being living entities continue to carry on the normal physiological processes resulting in the ripening and finally decay or death of the fruit even after they are separated from the parent plant/tree. Most of the fruits show the ripening changes after harvest with a few exceptions like grapes, which are to be ripened only on the vine, as they do not ripen well after harvest. Since the changes taking place in a fruit during ripening greatly influence the eating quality and the monetary value of the commodity depends on it, a detailed

knowledge of the physiology and biochemistry of ripening is desirable for the successful storage and marketability of fruits.

8.2 PHYSICOCHEMICAL CHANGES DURING GROWTH OF STORAGE ORGANS

Carbohydrate

Sugars are important for pleasing fruit flavour (sugar acid ratio) and texture. As the ripening starts these sugars undergo metabolic transformation both quantitatively and qualitatively. Most of the soluble carbohydrates are metabolized completely as the fruit ripens. Pectic substances and cellulose are the reserve carbohydrates that also serve as potential sources of acids, sugars and other respiratory substances during ripening.

Many changes occur in carbohydrate fraction of fruit during ripening, the climacteric and senescence. Green or raw fruit usually contains starch in abundance, but is short of soluble sugars that provides sweetness to it. During ripening, the starch is enzymatically [hydrolysis by alpha- and beta-amylases] converted into sugars. Thus, the major bulk of carbohydrate fraction of a fully ripened fruit consists of sugars. The sugars commonly found in fruits are glucose and fructose [invert sugars] and sucrose.

Organic Aacids

The organic acids are among the major cellular constituents undergoing changes during ripening. In most of the fruits there is a considerable decrease in the acidity of fruits during ripening.

The sourness of fruits is due to the presence of organic acids like citric, malic, succinic, tartaric, oxalic etc. These acids usually decide the quality of fruits as the blending of sugar and acids render the fruits tasty, besides flavour. Though these organic acids are present in varying amounts in raw or unripe fruits, but the concentration considerably changes as the fruits ripen. In fruits like oranges, the acids are converted enzymatically into sugars rendering them sweet as they ripen, whereas there is no change in lemons. So they remain sour till they start decaying. But, in some fruits like mangoes, there is a considerable decrease in acidity when the fruits fully ripen. This is partly due to the utilization of these acids in respiration through Kreb's Cycle. Generally, in fruits the total acidity shows a decrease with the increase in ripeness of the fruits.

Amino Acids and Proteins

A major turnover of amino acids in mango takes place during ripening, whereas in carombola shows a continuous decline. Small increase in protein content was also observed in mango, tomato and avocado.

The nitrogen content of fruit is due to proteins forming insoluble fraction and the soluble fraction comprised of amino acids. The total nitrogen content of fruits at the early stages is high, but with the advancement in growth, shows gradual decrease. This is probably due to increase in other constituents like

water, starch, sugar, organic acids etc. During ripening, the total nitrogen may show a further decrease in some cases.

Lipids

Phospholipids occur in the cytoplasm and in many structural units of plant tissues. They are physiologically more important than neutral lipids in storage organs. Considerable increases in the level of total lipids and fatty acids have been observed in ripening mango in contrast to many fruits and vegetables. However in fatty fruits of avocado the oil composition during maturation remains more or less constant.

Chlorophyll

Disappearance of green colour marks the initiation of ripening in most of the fruits. Chlorophyll content of ripening fruit decreases universally.

Carotenoids

A dramatic synthesis of carotenoids occurs during the last step of ripening. It has been reported that the levels of carotenes, free geraniol, mevalonic acid, all precursors of carotene biosynthesis increases progressively during ripening.

Other Pigments

The colour imparted to raw or ripe fruits and vegetables are due to presence of various pigments. The pigments of different tissues are the chlorophylls (green), anthocyanin [reddish to purple], flavonoids [yellow], leucoanthocyanins [colourless], tannins [colourless to yellow or brown], betalains [red], quinones and xanthones [yellowish] and carotenoids [yellow and red].

During storage, some of these pigments undergo considerable changes. Carotenoids formation and destruction may be affected by the storage conditions. In certain instances, these reactions are stimulated by O_2, inhibited by light and high temperature. Carotenoids include lycopene, alpha, beta and gamma carotenes are synthesized enzymatically in the fruits. Anthocyanin synthesis is stimulated by light and is often affected by temperature. Purple colour of red cabbage intensifies when stored below 10^{o} C. Chlorophyll degradation is accompanied by synthesis of other pigments as the fruits ripen. Chlorophyll metabolism is markedly influenced by environmental parameters, such as light, temperature and humidity and the effects of these factors are specific for the tissues. For example, light accelerates degradation of chlorophyll in ripening tomatoes and promotes formation of the chlorophyll pigment in cold stored potatoes.

Tannins

The tannins and other polyphenolic constituents are present in abundant quantities in immature, raw or developing fruits. As the maturity and ripening progresses the total polyphenolic content reduces gradually.

Pectic Substances

The most obvious change during ripening of fruit is the alteration in texture. The plant cell wall is made up of cellulose fibrils embedded in a matrix consisting largely of pectic substances, hemicellulose, proteins, lignins etc and water. Cell wall and middle lamella components increase during development of fruits, but as the fruit ripens the content of soluble pectates and pectinates increase, while total pectic substances decrease.

The cell walls are surrounded by parenchymatous cells which will absorb water and generate hydrostatic pressure within the living cells. This is called tugor pressure which gives the desirable property of crispness to the commodity. During storage, the loss of moisture due to transpiration and respiration results in the loss of crispness or the turgidity of the commodity. In addition, the changes in the pectic substances (which forms a component of the cell wall of the fruit cells) account for the firmness of the fruits. During ripening, the proto-pectin, which is insoluble and forms, the middle lamella of the cell wall, decreases in quantity and the soluble pectin content rises, thereby making the flesh less firm or soft. A decrease in the chain length and loss of methyl groups of proto-pectin probably occurs during ripening, accounting for the rise in soluble pectin. This is brought about enzymatically mainly by the activity of the enzymes pectinase and pectin methyle esterase.

Volatile Products

Each fruit has specific aroma which ripened fruit emanates. Although different fruits vary in nature of volatile compounds, they are emitted in noticeable amount only when the fruit starts ripening. Although the degree of maturity is the main physiological factor affecting aroma production, the aroma composition is also affected by environmental conditions during maturation. In overripe fruits mostly alcohol and esters are formed when fermentation develops.

One of the marked differences between an unripe and ripe fruit is the intensity of flavour of the fruit. The flavour of fruits or vegetables are considered to originate by the presence of basic constituents, such as carbohydrates (particularly mono- and disaccharides], proteins [particularly free amino acids] and fats [triglycerides or their derivatives], as well as vitamins and minerals. These constituents are produced through photosynthetic and related metabolic activities occurring in the commodities. Some volatile compounds may exist in the tissues as such but in some it may be formed enzyatically upon rupture of cells or by microorganisms. Besides ethylene, a number of other volatile odorous constituents like amyl esters of formic, acetic, valeric and caprylic acids have also been identified. These organic emanations produced during ripening of fruits contribute to the aroma of fruits and hence are of considerable importance from the standpoint of fruit quality.

Enzymes

Enzyme action is responsible for many chemical and physical effects during ripening. Softening of fruits, conversion of starch to sugar or vice versa, changes in amino acid content, and in colour.

Most of the biochemical changes occurring in fruits during ripening can be attributed to enzyme reactions. The change from starch to sugar, sucrose to invert sugar or protopectin to pectinic acid are all due to enzymic reactions.

Oxidative enzymes like catalase and peroxidase were shown to have increased to a considerable extent in 'Alphonso' and 'Neelam' varieties of mangoes during ripening as indicated by the higher rate of respiration. Similarly, glycolytic and hydrolytic enzyme activity was also found to increase in ripening mangoes, particularly during climacteric and post-climacteric period. Aspartie aminotransterase activity also increased in mangoes, resulting in the increased amounts of amino acids. Chlorophyllase activity followed the climacteric pattern in bananas, but suggested that the ensuing chlorophyll degradation may not be relevant to ripening. Other enzyme that increases in activity during ripening and following respiratory climacteric is fatty acid synthatase in Avocado fruit.

8.3 MECHANISM OF NUTRIENT MOBILIZATION AND ACCUMULATION

A developing commodity is the complex system of actively metabolizing tissue. Fruit growth generally starts by a short but rapid cell multiplication followed by cell enlargement. Initially cell division and enlargement contribute towards growth, but later cell division becomes major contributory factor. Large quantity of food is accumulated in storage tissue, the composition of which varies with the type of produce. Starch is stored in potato, garlic, banana; fat in avocado; malic acid in apple; citric acid in citrus and pineapple; ascorbic acid in guava; tartaric acid in grapes. Although the nature of chemical stored is a genetic characteristic, the mechanism of storage is physiologically controlled.

Concentration gradient is partly responsible for movement of nutrients from leaves to storage organs. Soluble carbohydrates from leaves are converted to insoluble carbohydrates in storage structure, thus create a gradient for further accumulation. Organic acids are contained inside the vacuoles. Once products are accumulated inside the organ some form of controls occurs to prevent their drain.

Some hormones are involved in the nutrient accumulation in storage organs. Stimulation of nucleic acid and protein synthesis by hormone treatment may cause translocation of nutrients, which establishes a physiological sink.

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 changes in carbohydrates take place during growth and ripening of the product?

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2. What is the effect of ripening on chlorophyll and carotenoids?

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3. What kind of food is stored in banana, apple, grape and guava?

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4. How carbohydrates are accumulated in tissues?

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8.4 RESPIRATION AND RESPIRATORY CLIMACTERIC

A major metabolic process occurring in harvested produce or in any living plant product is respiration. Respiration can be described as the oxidative breakdown of the more complex materials normally present in cells, such as starch, sugars and organic acids, into simpler molecules, such as carbon dioxide and water, with the concurrent production of energy and other molecules which can be used by the cell for synthetic reactions. Respiration can occur in the presence of oxygen (aerobic respiration) or in the absence of oxygen (anaerobic respiration, sometimes called fermentation).

Respiration rate of produce is an excellent indicator of metabolic activity of the tissue and thus is a useful guide to the potential storage life of the produce. If the respiration rate of a fruit or vegetable is measured as either oxygen consumed or carbon dioxide evolved – during the course of its development, maturation, ripening and senescence periods, a characteristic respiratory pattern is obtained. Respiration rate per unit weight is highest for the immature fruit or vegetable and then steadily declines with age. A significant group of

fruits that includes tomato, mango, banana and apple shows a variation from the described respiratory pattern in that they undergo a pronounced increase in respiration during ripening. Such an increase in respiration is known as a respiratory climacteric and this group of fruit is known as the climacteric class of fruits. The intensity and duration of the respiratory climacteric, varies widely amongst fruit species. The commencement of the respiratory climacteric coincides approximately with the attainment of maximum fruit size and it is during the climacteric that all the other characteristic changes of ripening occur. The respiratory climacteric, as well as the complete ripening process, may proceed while the fruit is either attached to or detached from the plant. Those fruits such as citrus, pineapple and strawberry that do not exhibit a respiratory climacteric are known as the non-climacteric class of fruits. Non-climacteric fruits exhibit most of the ripening changes, although these usually occur more slowly than those of the climacteric fruits. All vegetables can also be considered to have a non-climacteric type of respiratory pattern.

Respiration Rate

Respiration is the process by which stored complex materials are broken into simple products and energy is released. While respiration is essential to maintain the state of living of the produce, it causes deterioration due to losses in food reserves, food value, flavour, and dry weight. The rate of deterioration of harvested produce is generally proportional to their respiration rate. Respiration is linked to many quality parameters, which are responsible for spoilage. The rate of respiration is a good index of post-harvest life of fruits and vegetables as it is directly related to the rate of metabolism. According to respiration rate commodities can be classified as-

Class	Respiration rate (mg CO_2/ Kg/h at 5°C)	Examples
Very low	5	Nuts, dates, dried fruits and vegetables
Low	5-10	Apple, citrus, grape, garlic, onion, potato
Moderate	10-20	Apricot, banana, cherry, peach, plum, pear, cabbage, carrot, tomato, pepper
High	20-40	Strawberry, blackberry, cauliflower, lima beans
Very high	40-60	Snap beans, Brussels sprout
Extremely high	>60	Asparagus, mushroom, pea, spinach

Respiratory Climacteric

Many fruits and vegetables show a rapid increase in respiration during ripening and they are called as climacteric. Fruits that do not show such phenomenon are referred as non-climacteric. Although non-climacteric fruits are also reported to have rise in respiration rate with a concomitant rise in ethylene production or may show this effect at appropriate stage or under appropriate storage condition.

Factors affecting Respiration

A number of factors are responsible for variation in respiration rate. They include internal factors such as stage of development and chemical

composition of fruit tissues. External conditions also change respiration rate of the produce.

Stage of Development

During development as fruit size increases the total amount of CO_2 emitted by fruit also increases. In climacteric fruits, the respiration rate is minimum at maturity and remains rather constant, even after harvest. When ripening is about to start, respiration rate rises up to climacteric peak, then it slowly declines. Non-climacteric fruits ripen on tree, and if harvested early, a decline in respiration rate is observed. Actively metabolizing tissue has higher respiration rate. Small sized tissues will have higher respiration rate as they are having larger total surface area.

Chemical Composition of Tissue

The relationship between respiration rate and chemical composition varies among produce. Respiratory quotient varies with the type of substrate being used for respiration. It is 1 when substrate is sugar and less or more than 1 when lipids or organic acids, respectively, are the substrate. The level of moisture can also affect the respiration. Commodities with good natural coatings exhibit low respiration.

External Factors

Temperature: Respiration rate of fruits and vegetables increases 2-2.5 times for every 10°C rise in temperature.

Ethylene: In climacteric fruits exogenous application of ethylene advances the respiratory peak. In the non-climacteric fruits, respiration may be stimulated anytime during the life of the detached fruit, and an immediate rise in respiration occurs after ethylene application.

Oxygen and carbon dioxide: Rate of respiration increases with the increasing supply of oxygen, and carbon dioxide has an opposite effect.

Growth regulators: Depending on the time of application and quantity absorbed by the fruits, growth regulators may inhibit or stimulate the rate of respiration.

Fruit injury: Depending on the fruit variety and severity of bruising, injury can stimulate the respiration. This effect is indirectly attributed to ethylene production.

8.5 CLIMACTERIC AND NON-CLIMACTERIC FRUITS AND VEGETABLES

Climacteric fruits show a large increase in respiration rates and ethylene production as they ripen. In contrast non-climacteric fruits exhibit low CO_2 and C_2H_4 evolution rates during ripening. A non-climacteric fruit reacts to ethylene treatment at any stage of its pre-harvest or post-harvest life, whereas a climacteric fruit exhibit a respiratory response only if ethylene is applied

during the pre- climacteric stage, and it becomes insensitive to ethylene treatment after the onset of the climacteric rise Table 8.1.

Table 8.1: Some Examples of climacteric and non-climacteric fruits and vegetables

Climacteric	Non-climacteric
Apricot, banana, mango, avocado, cherry, peach, plum, pear, cabbage, carrot, tomato, pepper Apple, garlic, onion	Strawberry, blackberry, cauliflower, citrus, lima beans, Snap beans, Brussels sprout, grape, cucumber, pomegranate

8.6 MORPHOLOGICAL AND CHEMICAL CHANGES DURING RIPENING AND SENESCENCE

Compositional changes take place in harvested fruits and vegetables, which influence their colour, firmness, taste and aroma. During ripening there is change in colour of many products as the chlorophyll breakdown takes place, and new pigments are synthesized. Changes in carbohydrates include starch to sugar conversion and vice-versa, breakdown of pectins and other polysaccharides, which results in softening of fruit. Changes in organic acids and lipids influence flavour development. Synthesis of volatile organic compounds during ripening emits typical aroma of the commodity.

Many structural changes occur during ripening and senescence. In the sequence of events that leads to senescence of plant cell decrease in ribosome population and chloroplast breakdown are the first detectable symptoms.

Ribosomes

No change in ribosome population during maturation and ripening of tomato was observed as they were distributed throughout the cytoplasm and along the rough endoplasmic reticulum at all stages of fruit development, although a decrease was observed in post climacteric stage.

Mitochondria

A general degradative process is characterized by the reduction in the number of intact mitochondria, as senescence can be termed as the failure of the system responsible for keeping the cells in good repair. In the final stage of senescence, a shortage of ATP resulting from fewer active mitochondria can cause a loss of membrane integrity and redistribution of enzymes and substrate. In general mitochondria appears to be more resistant to breakdown than other organelles, and they persist till late stages of senescence.

Cell Wall

Differences occur in cell wall structure at different stages of fruit development. Loosening of cellulosic fibrillar structure is seen during ripening depending on the degree of solubilization of pectic and hemicellulosic substances between

the microfibrils. Thickness of cell wall changes and cells turn round and tend to dissociate.

Plastids

Plastids show more striking changes than all other organelles. As fruit matures, starch granules disappear and the osmophilic granules increase in number and size. With maturity grana disappear and structure similar to thylakoid plexes are seen. In ripe stage, chromoplast development takes place with granal lyses and increase in size and/or number of lipid granules. Generally chlorophyll disappears from senescing plant tissue with degeneration of granal lamellae, formation of a single membrane system, and an increase in size and/or osmiophilic globules.

Intracellular Spaces

Intracellular spaces are formed by separation of cells along the middle lamella. A decrease in porosity is observed with ripening.

Other Cell Organelles

The endoplasmic reticulum vesiculates and disappears with senescence. Golgi apparatus also disappears and tonoplast breakdowns with senescence. The nucleus and plasmalemma are the last structure to vanish and it brings about the death of cell.

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. How developmental stages affect the respiration rate of the product?

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2. How chemical changes taking place during ripening affect rate of respiration?

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3. What changes in mitochondria occur during ripening and senescence of the produce?

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4. What are the cell wall changes during ripening and senescence of the produce?

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8.7 LET US SUM UP

Taste of fruits and vegetables is associated with the amount and type of chemical constituents and the physical nature of the commodity at harvest. During ripening a series of changes in colour, texture and flavour are evident. These changes are influence by respiration and ethylene production by the produce. It is important to realize that no improvement in quality can be done in post harvest stage; only efforts can be made to keep intact the quality attained at harvest.

8.8 KEY WORDS

Physiology : Study of the functions and vital processes of living plants.

Ethylene : A colourless flammable gas which stimulates ripening.

Ripening : The advance stage in the development at which fruit and vegetable are suitable for consumption/ utilization.

Climacteric : Fruits/vegetables showing a sudden upsurge in respiration coupled with ethylene evolution.

Non-climacteric : Fruits/vegetables who do not show a sudden upsurge in respiration coupled with ethylene evolution.

Respiration : Process of inhaling oxygen and exhaling carbon-di-oxide.

Development : A process of growth towards more perfect stage.

Maturation : Becoming full grown or fully developed.

Senescence : Beginning of final phase in the life of plant.

Volatiles : A substance having the quality of gas.

Pigments : Colouring matter in the cells or tissue of fruits and vegetables.

Aroma : A smell coming out of the product.

Fermentation : The breaking down of complex organic compound by microorganisms.

Coating : Layering of the outer surface of fruits or vegetables.

Bruising : To injure the surface without breaking the skin, but causing Discoloration.

8.9 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answers should include following points:

   - Pectic substances
   - Starch

2. Your answers should include following points:

   - Chloroplast
   - Chromoplast
   - Mevalonic acid
   - Chlorophyllase

3. Your answers should include following points:

   - Starch
   - Acids

4. Your answers should include following points:

   - Insoluble carbohydrates
   - Gradient

Check Your Progress Exercise 2

1. Your answers should include following points:

   - Fruit size
   - Size and metabolic stage of tissues

2. Your answers should include following points:

   - Sugars
   - Organic acid
   - Lipoids

3. Your answers should include following points:

   - More resistant
   - Membrane integrity

4. Your answers should include following points:

   - Cellulosic fibrillar
   - Pectin solubilization

8.10 SOME USEFUL BOOKS

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UNIT 9 PHYSIOLOGICAL DISORDERS

Structure

9.0 OBJECTIVES

After going through this unit, you should be able to:

- know the reason for different physiological disorders;
- mention the symptoms of major physiological disorders;
- state the importance of minerals in fruit quality; and
- tell how the occurrence of physiological disorders can be avoided.

9.1 INTRODUCTION

Physiological disorders are related to exposure of undesirable environmental condition such as temperature, very low oxygen or high carbon dioxide, humidity and nutritional disorders etc. In general any breakdown of tissues other than invasion of pathogens or mechanical damage is termed as physiological disorder.

Different fruits and vegetables have different tolerance limits to cold (low) temperature, storage below which the stored commodity is susceptible to various kinds of disorders including fungal infections.

9.2 PHYSIOLOGICAL DISORDER OF TROPICAL AND SUB-TROPICAL PRODUCE

Many tropical and sub-tropical varieties of fruits and vegetables when stored below 10°C for a longer time suffer physical and physiological injuries. These injuries are of the following types:

i) Superficial scald
ii) Carbon dioxide injury
iii) Core flush

iv) Breakdown of the flesh of the stored commodity:
   a) Low temperature breakdown [LTB]
   b) Senescent breakdown
v) Water core
vi) Bitter pit
vii) Freezing injury
viii) Chilling injury

1. Superficial scald

The common feature of this is that the areas of the skin of the stored commodity turn brown. These areas are very slightly sunken and the lenticels look as injured spots, e.g. apples, pears, and peaches.

2. Carbon dioxide injury

Usually occurs in CA storage when carbon dioxide concentration goes higher. The periphery of the internal tissue turns slightly brown initially and as the time of exposure to CO_2 prolongs the tissue turns deep brown, e.g. apples, pears, mangoes.

3. Core flush

Core flush is yellowish-pinkish discolouration of the core of the apples. It may appear as a ring of damaged tissue or it may involve the whole area of the core.

The incidence of the core flush is aggravated by increased CO2 concentrations in the core atmosphere. Storage in the absence of CO2 and in low concentration of oxygen gives good control of core flush.

4. Low temperature breakdown [LTB]

Low temperature breakdown of apple is seen in the cortical tissue as a general browning of the flesh that can vary in intensity from season to season.

As the disorder progresses the skin becomes discoloured and water logged, giving a dark translucent appearance. The cut surface looks moist.

5. Senescent breakdown

Senescent breakdown of apples and pears is a disorder associated with over maturity and it develops further at high temperatures when the fruit is removed from store. It is variable in appearance but fruit looks drier as compared to low temperature breakdown. Sometimes the flesh also becomes mealy.

6. Water core

Water core is a condition in which parts of the flesh of the commodity appear to be translucent and glossy because the intercellular spaces have become injected with the sap. It is more prominent in the flesh and may

also appear near to the surface of the skin. Water core disappears rapidly at higher storage temperatures, but badly affected fruits cannot recover from water core disorder.

7. Bitter pit

Bitter pit is appearance of small brown dry areas on the skin, which also disfigures the flesh. The location of the pit is usually below the skin, but in severe cases the pits may extend right up to the cortex. Under the microscope the pitted areas are seen to consist of dead collapsed cells. Deficiency of calcium in the soil causes bitter pits, and it can be avoided by pre-harvest spray of calcium. The bitter pit mainly occurs in pears, apples, and guavas.

8. Freezing injury

Freezing injury occurs when storage temperature falls below 0°C. The affected fruit externally has an irregular shape caused by tissue collapse, and the juice streams out of the injured or cut tissue even under slight pressure.

In apples, freezing injury characteristically occurs in cone-shaped segments with the apex at the core.

9.3 LOW TEMPERATURE DISORDER – CHILLING INJURY

Chilling injury is a major problem in post harvest handling of fruits and vegetables. This injury occurs at temperatures which are lower, but much above the freezing point of the tissues. Chilling injury is manifested in a variety of symptoms such as surface pitting, discolouration, appearance of water soaked areas, increased susceptibility to decay, loss of sprouting ability, etc. Some of the important physiological responses to chilling injury are stimulation of ethylene production, and failure of colour development.

Chilling injury is a phenomenon during which many tropical and subtropical fruits and vegetables when stored below 10°C develop the symptoms of skin discolouration and browning, pitting of the skin, water soaked spots, soggy flesh and failure to ripen when the commodity is removed to room temperature [RT].

The chilling injury symptoms are visible only after 2 or 3 days storage at room temperature.

9.3.1 Control of Chilling Injury

Temperature Pre-conditioning

Gradual reduction of storage temperature of the cold room is found beneficial in alleviation of CI. Pitting in stored banana was found reduced from 90.6 to 8.9% when stored at 13°C-12°C-11°C-10°C at 4 days intervals.

Regulating Humidity

Storage humidity at 95-100% was found to reduce CI symptoms in bananas stored at 11°C when they are covered in polythene bags.

Intermittent Warming

Intermittent warming [IW] of the commodity for every 5 days at low temperature/2 days at Room Temperature was found effective to control CI. Raw green mature papaya stored at 7°C for 5 days when transferred and kept for 2 days at RT did not exhibit CI symptoms for 3 weeks storage. Similarly, green mature bananas responded to this treatment with control of CI symptoms when removed to RT after 3 weeks I.W. at 8°C.

Wax Coatings

Suitable concentrations of wax coatings of the skin of papaya, banana and mango stored at 8°C for 2 weeks did not develop CI symptoms when removed to RT.

Modified Atmosphere Storage [MA Storage]

When the fresh commodity is enclosed in the thin low density polyethylene (LDPE) bags and stored at chilling temperature, the commodity stored, resisted CI up to 2 weeks. Banana, papaya and mango when enclosed in sealed polythene bags develop modified atmosphere and humidity inside the bag, which helps to alleviate CI symptoms. The fruits when removed to RT after 2 weeks of storage at chilling temperature and 8°C did not show CI symptoms.

9.3.2 Chilling Injury Symptoms

Fruit	Safe storage temperature (°C)	Symptoms
Banana	12	Brown streaking on screen
Cucumber	7	Dark coloured water soaked areas
Brinjal	7	Surface scald
Lemon	10	Pitting, red blotches, membrane staining
Mango	12	Dull skin, brown areas on skin
Papaya	7	Pitting, water soaked areas
Pineapple	10	Brown or black flesh
Tomato	12	Pitting

9.4 HIGH TEMPERATURE DISORDERS

Exposure to high temperature or direct sunlight can cause bleaching, scalding, uneven ripening and desiccation in horticultural produce. Sunburn scald in apple is an example of high temperature disorders and its symptoms vary from brown to black areas damaged by sunlight.

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 chilling injury?

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2. What are the symptoms of chilling injury in lemon and mango?

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3. What are the general symptoms of high temperature disorders?

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4. What is sunburn scald of apple?

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9.5 DISORDERS DUE TO ALTERED ATMOSPHERIC COMPOSITION

Although modification of atmosphere around produce is done to extend the shelf life of horticultural commodity, they are very sensitive with regards to the concentration of carbon-di-oxide and oxygen. High carbon-di-oxide levels cause uneven colour development and excessive softening in tomatoes. Reduced oxygen levels cause internal browning of pears. Some physiological disorders of fruits are shown in Table 9.1.

Table 9.1: Physiological disorders of fruits

Product	Disorder	Symptoms
Apple	Superficial scald	Sunken skin discolouration
	Sunburn scald	Brown to black area damaged by sunlight
	Senescent breakdown	Brown, mealy flesh
	Low temperature breakdown	Browning in cortex
	Water core	Translucent areas in flesh
	Brown heart	Brown areas in flesh
Pear	Core breakdown	Brown, mushy core
	Neck breakdown	Brown to black discolouration of vascular tissues connecting stem to core
	Superficial scald	Grey to brown skin speckles
	Brown heart	Brown areas in flesh
Grape	Storage scald	Brown skin discolouration
Citrus	Storage spot	Brown sunken spots on skin
	Cold scald	Superficial grey to brown patches
	Stem end browning	Browning of shrivelled area near stem end
Peach	Wooliness	Red to brown dry areas in flesh

9.6 MINERAL DEFICIENCY DISORDERS

Plants require a balanced mineral intake for proper development; if any of the essential minerals are deficient it will be manifested during plant development or in the plant or plant parts. Many times the browning symptoms in certain fruits and vegetables are attributed to the deficiency in some mineral constituents of the produce. These disorders can be prevented by either pre-harvest or post harvest application of specified minerals.

Calcium deficiency is one of the major problems. Calcium is physiologically important mineral as its deficiency may suppress respiration and several other metabolic sequences in plant tissues. Further, calcium is associated with pectic

substances in the plant cell that helps in strengthening of tissue. Potassium is other mineral that manifests its symptom both during excessive application and deficiency. Other minerals may also play role in maintaining proper plant and produce health. Toxicity of copper, iron and cobalt may cause similar symptoms to low temperature break down and superficial scald in apples. Copper also acts as a catalyst to browning enzymes (Table 9.2).

Table 9.2: Mineral deficiency disorders in fruits and vegetables

Fruit	Mineral	Disorder
Apple	Calcium	Bitter pit, cork spot, cracking, low temperature break down, senescent breakdown, water core
	Boron	Internal cork
	Potassium (High)	Bitter pit
Beans	Calcium	Hypocotyls necrosis
Cabbage	Calcium	Internal tipburn
Carrot	Calcium	Cavity spot, cracking
Mango	Calcium	Soft nose
Pear	Calcium	Cork spot
Pepper	Calcium	Blossom end rot
Potato	Calcium	Tipburn
Tomato	Calcium	Blossom end rot, cracking, black seed
	Potassium	Uneven ripening

Blotchy Ripening, Greenback or Solar Yellowing of Tomatoes

It is green, yellow or translucent hard patches of tissue spread over the red colour of ripe tomatoes. The parenchyma surrounding the vascular bundles of the outer fruit walls are necrotic and disorganized. The affected tissues may either be opaque or brownish in colour and are lignified and starchy.

The green areas of blotchy fruit contain less solids, nitrogenous compounds and sugars; more total and insoluble pectic substances; less pectinesterase and polygalacturonase activity; and lower titratable and total acids than the red parts.

Blotchy ripening of fruit may be affected by the nutrition of the plant. Potassium deficiency or excess nitrogen nutrition may bring about more blotchy fruits. Blotchy fruit may be not only a nutritional effect but also a result of stress leading to tissue compartmentalization. The blotchy section has different metabolic pattern than the normal tissue development.

Shading fruit with aluminium foil caused a decrease in the severity of greenback, whereas shading with black PVC foil increased its occurrence. Green back is caused by variations of temperature on the tomato fruit pericarp during maturation. The higher the temperature at any part of the fruit, the

greater the incidence of greenback. Temperature is a direct effect of incoming radiation and heat transfer coefficient. Factors such as variation in the chlorophyll content around the fruit, position of the fruit relative to the sun, stages of maturity, size and variety will produce localized temperature differentials. Metabolic activity would likewise differ, leading to blotchy ripening.

It is commonly observed that large fruits are more frequently and severely damaged by greenback than small fruits. The intensity of greenback depends on length of exposure and degree of temperature. Defective colouration of tomato shoulders is not primarily a heat effect but mainly a result of short-wave radiation. Short-wave radiation not only reduces the carotenoid synthesis in blotchy fruits, but also inhibits the C2H4 production.

Blossom-end Rot

This disorder is described as brown proteinaceous inclusions occurring in the epidermis and pericarp, at the stylar-end of the fruit. Cell membranes become disorganized and tissue necrosis develops underneath, the skin remaining intact. The causal factor for blossom end rot may be the Ca deficiency. This can be effectively reduced by application of Ca $(NO_3)_2$ and gypsum or by spraying with $CaCl_2$ solution. Excess K may also result in the production of blossom-end rot, probably through a Ca deficiency-induced effect. Water stress may aggravate this disorder.

Cuticle Cracks

Shallow, slightly dark, tiny but well healed cracks on tomato fruit surface are referred to as cuticle cracks or skin checks. Because the waxy covering is removed, water loss is rapid and shrivelling and discolouration follow. Cuticle crack is probably caused by the fluctuations in moisture regime and temperature.

Fruit Tumor or Waxy Blister

This disorder consists of a wax-like irregular tumor on the fruit surface starting as smooth, turgid blisters, which turn brown, depressed and cracked as the fruit ripens. Blisters may be induced by rubbing green fruits and storing at 20-35°C. The injury caused by rubbing apparently sparks the synthesis of more growth hormones with kinin like activity, which causes increased cell division and result in tumorous growth. Blister is mainly a handling disorder and may be controlled by careful picking, proper packing and minimizing damage during transport. Ripening at lower temperature is also recommended for avoiding this disorder.

Growth Cracks

This disorder is characterized by rupturing or cracking of the fruit, usually either around the stem-end (concentric) or from the stem-scar down the fruit shoulders (radial). Abundant rainfall and high temperatures favours rapid growth and predispose tomato fruits to growth cracks. The specific cause of rupturing could be an uncoordinated tissue expansion during growth or simply

a turgidity phenomenon. It may be controlled by picking before the fruits are ripe and by planting crack-resistant varieties.

Puffiness

Puffy tomato fruits are downgraded or are rendered unmarketable in serious cases. Affected fruit is hollow and light in weight. The surface between the internal cross walls is usually flattened or sunken. The large pockets are observed in cut tomatoes in the cavities occupied by the seed-bearing tissues.

Factors, which inhibit normal pollination, may cause poor development of seed-bearing tissues, and growth in such fruits lags behind the normally developing peel tissues leaving empty spaces between them. A too high or too low growing temperature, drought, excessive moisture supply and heavy N application interferes with normal pollination and hence should be avoided to reduce the incidence of puffy fruits.

Sunscald or Sunburn

Cabbage: Sunscald in cabbage starts as blistered, irregularly shaped areas that become papery and bleached later. Exposed leaves on the top of the head are usually affected. Because of possible secondary infection by decay-causing microorganisms, affected leaves should be removed prior to packing.

Pepper: The sunscald in pepper is due to exposure of the fruit to the intense heat of sunlight. Sunburn appears as light-coloured and soft areas on the fruit surface, which eventually becomes papery. Another type of injury, termed delayed sunscald, which appears after harvesting. Initially the fruit is water soaked and becomes dry and brown, but lacks the bleaching and papery symptoms.

Pomegranate: Exposure to the sun during fruit growth produces a brown, tough, leathery and slightly russetted patch on the rind.

Beans: Beans affected by sunscald appear first as tiny reddish spots forming reddish-brown streaks across the pods. At an advanced stage of sunscalding, pods become water-soaked followed by browning and shrinking of the affected tissue. This occurs only on one side of the pod, and is more serious during moist weather.

High Temperature – Induced Desiccation

Avocado (Heat Injury): Prolonged exposure of some cultivars of avocados to a temperature of 20°C causes damage to the fruit and the fruits held at 25°C to 30°C will not ripen normally. It causes uneven softening and discolouration may occur which makes fruit unpalatable due to off-flavours. The flesh darkens and brown spots appear on the skin. At 32°C, the flesh becomes rubbery and pitting like symptoms on the skin may occur.

Banana (Dehydration): Water loss in banana results in shrinkage of tissue or may even cause symptoms similar to severe chilling. Pitting in bananas may be induced either by high temperature or low RH. RH below 80% may produce symptoms characteristic of low-temperature breakdown. These effects lead to

abnormal ripening. Therefore to avoid dehydration, bananas should be promptly cooled after harvesting and stored at humidities between 90 and 95%.

Lychees (Browning): Lychee browning is a desiccation phenomenon due to exposure to dry air. It starts at tubercle tips, creeping downward and spreading on the bright-red shell. At an advanced stage, the entire surface of the fruit may turn completely brown. Browning may be reduced by storing at 2°C for up to 5 wk or at 7°C for less than 2 wk in polyethylene bags.

Onions (Translucent Scales): Translucent scale of onions consists of clearing of the normally opaque cells due to a disintegration of the parenchyma walls. It looks similar to freezing injury, however the distinguishing features are mentioned below:

	Freezing Injury	**Translucent Scale**
Pattern of damage	From the surface inward	No pattern
Stem plate	May be affected	Not affected
Scales	Outer more affected than inner	Inner may be more affected than outer
Freshly cut surface	Dry	Moist
Areas of white opaque tissues	Present	Lacking
Epidermis of affected scales	Loose	Loose only in severe cases
Texture of surface with epidermis removed	Grainy, rough	Smooth, slick

Major factors influencing the severity of this disorder include delays at 15 to 30 days between the end of curing and the start of storage at 0°C during 7 months storage. Tropical conditions where the temperatures goes above 32°C for about 50% of the growing period or above 35°C for about 30% of growth period of the plant, have high probability of developing this disorder during storage. Prompt cold storage and early covering the bulbs with soil reduces the incidence.

Growth Cracks and Splitting

Pomegranate (Splitting): Cracking of pomegranate during ripening is a natural characteristic believed to be due to humidity fluctuations, dry winds and irrigation. Picking over-mature fruit should be avoided to prevent cracking during storage. Cracked fruit may serve as an avenue for entrance of decay-causing organisms

Sweet potatoes (Growth cracks): Fairly deep fissures may develop on sweet potatoes due to successive growth interruptions in the field. In some cultivars, high N fertilization and irrigation followed by a dry weather may induce growth cracks.

Disorders Related to Certain Field Conditions

Carrots (Scab Spot complex): Early signs of scab spots of carrots are pockets of black necrotic tissues occurring usually at or near the lateral rootlets, which later become sunken and scab-like. The cause of this disorder could be related to nutritional, climatic and genetic factors.

Potatoes (Surface Browning): Under conditions of low RH and high harvest temperatures, some portions of mechanically injured tubers undergo oxidation, resulting in objectionable surface browning. The affected area darkens with time and becomes much more noticeable after a few days. Surface browning may be induced at RH ranging from 25 to 30%. Temperature is secondary to RH as a factor in browning, but higher temperatures are associated with low RH, thus compounding the predisposition of tubers to oxidation.

Mango (Black-tip): Orchards near the brick kiln suffer heavily every year due to black tip necrosis of mango. Small-etiolated area at distal-end of the fruit appears after 3 to 4 days of fruit setting. It gradually increases in size and the tip becomes necrotic, often exposing the stone of the fruit as a result of disintegration of outer tissues. Affected fruits do not mature properly and tip becomes hard and black.

Regular sprays of boron from the flowering stage can control this disorder. Spraying mango tree with aqueous solutions of NaOH and Na_2 CO_3 minimizes losses due to black-tip.

Pineapple (Endogenous brown spot or black heart): This physiological disorder in its early stages is characterized by the formation of watery spots at the base of fruit lets near the fruit core. With increase in severity of the disease, the spots enlarge and turn brown; with further increase in severity, the spots turn darker and may join together to form a dark mass in the centre of the fruit.

It can develop in the fruit if the pineapples are chilled at low temperatures. There are no visible symptoms of the disease in uncut fruit either before or after the onset of the spots in the pulp, thus making it impossible to remove affected, fresh fruit prior to shipment. Hence, losses in commercial surface shipments of refrigerated fruit have been high, but no losses occur in non-refrigerated air shipments.

Storage Disorders

Onions and other commodities (Ammonia injury) Accidental exposure of onions to ammonia during cold storage may bring about marked discolouration. Red onions change to blackish-green and then dull greenish-black later. Yellow onions show initial yellowish-green colour on the edges of scales, turning bronze to brownish-black at an advanced stage. White onions become greenish-yellow on exposure to ammonia. Discolouration is always much more rapid and pronounced in a more humid atmosphere. Exposure to a 1% ammonia vapour for one hour is sufficient to initiate discolouration. Exposure to higher concentration of ammonia from leaks in cold storage may bring about colour changes almost immediately, and brownish-black areas may be seen within a few minutes.

Other Fruits and Vegetables are also Injured by Exposure to Ammonia

Banana	-	brown to black and tissue breakdown;
Grapes	-	discolouration of berry and complete breakdown of the tissue;
Citrus (sweet orange and lime)	-	dark brown discolouration of the rind;
Mango	-	brown surface, pitting and breakdown of the tissue;
Potato	-	brown to dark brown pock marks, pitting, internal discolouration and watery breakdown
Tomato	-	impaired colour development, discolouration of the skin and breakdown of the tissue.

Potatoes (Greening): Greening of potatoes exposed to light during storage occurs due to synthesis of bitter, toxic alkaloid solanine. Although chlorophyll formation is independent of solanine synthesis the same factors, i.e., light quality and intensity, storage duration and age of tuber, affect solanine formation.

Sweet potatoes (Internal breakdown): Internal breakdown is a storage disorder where the internal tissues of sweet potatoes are pithy, dry and spongy. This usually occurs late in the storage season in warm and dry storage rooms, or in rooms with chilling temperatures.

CA Storage Disorders

Two types of disorder may be developed under a CA storage condition: injury due to sub oxidation and that due to CO_2 accumulation. Other volatiles may accumulate in the storage rooms above the critical level resulting in the development of off-flavours and off-odours and progressive death of the tissue.

Sub-oxidation causes blackheart in potatoes, promotes browning in limes, or produces off-flavour and objectionable alcoholic odour in many fruits and vegetables. High CO_2 on the other hand, (a) produces a slight carbonated taste in melons; (b) a general browning of surface (e.g. asparagus) and internal tissue (e.g. cabbage); (c) a stimulation of ethyl acetate production on strawberries (d) off-flavour; (e) off-odour; (f) pitting; (g) impaired ripening; (h) susceptibility to decay-causing microorganisms; and (i) mild necrosis in some fruits and vegetables.

Disorders of Uncertain Causes

Escarole (Marginal browning)

Edges of leaf blade become dry, dark, curly and brittle. The control measures include refrigerated transit, adequate refrigeration, prompt pre-cooling and marketing, removal of old leaves and discarding over-mature escarole may minimize the occurrence of marginal browning

Garlic (Waxy breakdown)

Garlic bulbs are seriously affected by a yellow waxy breakdown believed to be a physiological disorder. The flesh of the clove is somewhat sticky or waxy to the touch but not disintegrated. The outer scales show no indications of breakdown.

Lettuce (Marginal browning: Pink rib and russet spotting complex)

Marginal browning is a physiological disorder characterized by yellowing, followed by browning or necrosis of wrapper-leaf margins. Adverse growing conditions or improper transit and storage conditions, which accelerate senescence, appear to be the origin of this disease. Control measures include refrigerated transit, adequate refrigeration, prompt pre-cooling and marketing, removal of old leaves and discarding over-mature lettuce may minimize the occurrence of marginal browning.

Heads of lettuce affected by pink rib have pinkish, wrinkled and pebbly textured midribs. Over-mature lettuce or plants held long in storage show higher incidence of pink rib. The causative factor is not known. The control measure includes the measures adopted to delay senescence.

Russet spotting complex includes several types of discolouration originating from the field, transit and storage, but distinction among them is uncertain. These symptoms include russet, vein browning, C_2H_4 burn, red heart, internal browning, brown spot, brown blight, rust or storage breakdown. In general, the variable symptoms are irregularly shaped specks ranging in colour from light yellow, pink to dark brown, affecting the ribs, veins and interveinlet tissues. Because of the complexity of origin, only routine quality control measures may help to ameliorate the disorder. Thus, harvesting properly matured heads, rigid grading and culling at handling points, use of refrigerated trucks during transport and prompt pre-cooling and marketing may reduce its incidence.

Mango (Internal breakdown): This physiological disorder is most common in ‘Alphonso’ mango. It is termed as ‘internal breakdown’, ‘spongy tissue’, or ‘soft centre’. So far it is observed only in this cultivar. Externally, the fruit appears to be sound. The disorder is noticed when the fruit is cut into halves. It is observed only in semi-ripe and ripe fruits. The breakdown tissue is characterized by pale yellow colour, soft or spongy texture with or without off-flavour. It starts from the tissue adhering to the stone and gradually spreads to the periphery. In extreme cases, the whole flesh portion becomes too soft resembling bacterial rot. The causative factors for the onset of this breakdown and its control are not known.

Pomegranate (Internal breakdown): Internal Breakdown of pomegranate is characterized by arils, which become light in colour; flat in taste; and sticky in appearance. White lines radiate in all directions from the seed to the outer wall or aril.

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. How high carbon dioxide and low oxygen are detrimental to fruits and vegetables?

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2. Explain the importance of minerals in quality of horticultural produce?

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3. Explain the role of calcium in avoiding certain physiological disorders.

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4. How copper is helpful in maintaining quality of produce?

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9.7 LET US SUM UP

Different advance techniques are used during production as well as storage of products. It is important to use these techniques properly so that spoilage of product due to physiological disorders can be avoided. Preventing the metabolic sequences that leads to the development of disorder can prevent disorders. Sometimes chemicals are used to prevent the disorders. Genetic improvement of horticultural cultivars may also help in alleviating the occurrence of disorder. Physical methods of maintaining optimum storage temperature and storage atmosphere may help in reducing many disorders.

9.8 KEY WORDS

Disorders : Not a normal growth or product.

Symptoms : The condition that accompanies something and indicates its existence.

Nutritional : Substances that promote growth.

Ethylene : A colourless flammable gas which stimulates ripening.

Browning : To become dark or surface develops brown pigments.

Deficiency : Absence of some essential thing forms the nutrition.

Toxicity : Consumption of which acts as a poison.

Genetic improvement : Improvement of quality of a product by using the improved hereditary material.

9.9 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answers should include following points:

   - Low temperature storage
   - Surface pitting
   - Ethylene production
   - Uneven ripening

2. Your answers should include following points:

   - Pitting
   - Dull skin
   - Skin discolouration

3. Your answers should include following points:

    - Bleaching
    - Scalding
    - Desiccation

4. Your answers should include following points:

    - High temperature disorder
    - Sun scorching

Check Your Progress Exercise 2

1. Your answers should include following points:

    - Softening
    - Improper colour development

2. Your answers should include following points:

    - Proper development
    - Deficiency manifested in form of disorders

3. Your answers should include following points:

    - Pectic substances
    - Deficiency suppresses respiration

4. Your answers should include following points

    - Catalyst
    - Deficiency causes disorder

9.10 SOME USEFUL BOOKS

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UNIT 10 FERMENTATION, METHOD OF FERMENTATION AND INDUSTRIAL SIGNIFICANCE

Structure

10.0 OBJECTIVES

After studying this unit, you should be able to:

- state the meaning of biotechnology and fermentation;
- infer the historical importance of different varieties of fermented foods prepared all over the world from various agricultural commodities;
- know the nutritional significance and beneficiary effects of fermented foods and their classification;
- describe the general methods in fermentations for the cultivation of microorganisms;
- state the importance of aerobic and anaerobic fermentations; and
- discuss the difference between solid state fermentation and submerged fermentation.

10.1 INTRODUCTION

In recent years, biotechnology and information technology have been given much importance. It is believed that these areas of Science and Technology would help to improve the standard of living as well as the life span all over the world. Biotechnology is the integrated use of biochemistry, microbiology and chemical engineering to achieve industrial application of microorganisms and tissue culture. Unlike material sciences, in biotechnology the key of any process or activity is the biological source viz., microorganisms (bacteria,

yeasts, fungi and virus) plants and animals. If we scan the books and journals, we find most of the processes are based on microbes through fermentation. These microorganisms produce newer types of products (vitamins, antibiotics, hormones, amino acids, enzymes, beverages, fuels and solvents, life saving drugs) or convert raw food stuffs (cereals, pulses, fruits, vegetables, animal, fish, poultry and dairy products) into palatable, nutritious and processed foods with longer shelf life.

Fermentation is the oldest word used for biotechnology from time immemorial when nothing was known about microorganisms or technology. Bread-making, cheese manufacture and production of wine and alcoholic beverages are the certain examples. *Fermentation can be defined as the chemical modification of organic compounds or raw materials or agricultural commodities with the aid of enzymes produced by microorganisms*. With respect to foods, it is the transformation of physical structure and chemical constituents (carbohydrate, proteins, fats and nucleic acids) by microorganisms and their enzymes under aerobic (presence of oxygen) or anaerobic (absence of oxygen) conditions. Examples are wine from grapes, cider from apples, beer from malted barley, whisky, gin, rum, pickles, curds, idli and dosai, bread and nan, cheese and lactic beverages.

10.2 HISTORY OF FOOD FERMENTATIONS

When we go through several books and research articles we find a number of definitions of fermentation. The most frequently used names were: respiration, fermentation, putrefaction, decay, digestion, dissimilation and ferment. However, the major developments in the twentieth century in the field of microbiology, molecular biology, chemical engineering and biochemical engineering and biochemistry have resolved this confusion.

If we look at the human's civilization, the man has passed through several stages viz., hunting, gathering food to this stage of collective cultivation and processing of foods. During the early stages, sun-drying, salting and fermentation were practiced and in certain pockets especially in tribals and rural areas in India and other countries, these are in vogue sometimes singly or in combinations.

The Egyptians, Sumarians, Babylonians, Assyrians and Indians knew the technique of alcoholic beverages production. Indians were used to *Ashwas* and *Arishtas*. Drinking wine was common in the Roman Empire throughout Europe and North Africa. Mead was prepared from honey. Idli, dosai and other fermented foods were introduced in South Indian dietary system since time immemorial. Soy sauce, miso and tempeh were known to Chinese as early as 1000 BC and the techniques were passed on to Japan around 600 AD. Fermented dairy products such as curds and butter milk are referred in early Sanskrit literature. The word fermentation comes from the Latin word *ferveo* meaning to boil. From this *fermentum* was derived which means yeast or leaven. The evolution of bubbles due to carbon dioxide production was noticed in alcoholic fermentation and hence this name was given. Louis Pasteur in France in 1861 was first to declare that a group of microorganisms viz., yeasts are responsible for fruits and grains fermentation for the manufacture of wine and beer. He also described that along with desirable type of microorganisms, undesirable types also grew in the ferment and also these were responsible to spoil the quality of final product. He was first to suggest heating at 62.8^{o}C for

30 minutes (pasteurization) to eliminate the undesirable microorganisms from the fruit juice before fermentation.

10.3 MICROBIOLOGY AND BIOCHEMISTRY

As you have become aware, food fermentations started with the development of man's civilization purely due to necessity. During those days nothing was known about science and technology of fermentation and also causitive agents viz., microorganisms and their enzymes responsible for it. It was also not known that these microorganisms fall under two categories: **useful** producing life saving drugs, chemicals and fermented foods and products and **harmful** causing spoilage of the products and responsible for many deadly diseases (Cholera, typhoid, plague, small pox etc.). These became evident after the discovery of microorganisms and their systematic scientific studies in the middle of nineteenth century when Louis Pasteur established their role in fermentations and human and animal diseases. In India, food prepared if left over night outside, gets spoiled due to the growth of microorganisms. Microorganisms spoil the food by damaging the structure, colour, and chemical and physical characteristics both in raw as well as processed foods. Bread (*Rhizopus nigricans, Aspergillus niger, Penicillium notatum)* fresh fruits and vegetables (*Rhizopus* and *Erwinia)* Pickles (*Rhodotorula, Candida, Pichia),* meat (*Alcaligenes, Clostridium, Proteus vulgaris, Pseudomonas fluorescens),* fish (*Flavobacterium, Alcaligenes)* eggs (*Pseudomonas fluorescens)*, orange juice (*Lactobacillus, Leuconostoc, Alcaligenes)* and poultry (*Pseudomonas, Alcaligenes)* are spoiled by a number of microorganisms and thus become unfit for consumption.

Some bacteria such as *Salmonella* spp., *Shigella dysenteriae, Vibrio cholerae, Clostridium botulinum, Clostridium perfringens, Listeria* spp. and *Campylobacter* spp. produce toxins causing food poisoning. *Aspergillus flavus, Pencilllium citrinum, Fusarium graminarium* and many strains and species of fungi in under humid and hot conditions grow on agricultural commodities and food materials and produce different types of toxins which are deleterious to human and animal health.

The growth and activity of microorganisms thus are important in controlling the quality and safety of food. Both harmful and useful microorganisms grow in their environmental and ecological conditions in food commodities, food preparations and processed foods and other systems and produce enzymes, metabolites and toxins depending on nutritional and physical factors. These factors are water activity, pH, temperature, chemical constituents and the buffering compounds. In natural fermentation (without addition of microorganisms, inoculum or starter culture) and controlled fermentation (addition of starter culture/s), microorganisms bring about many chemical and structural changes in organic constituents of foods and thus check the growth of pathogenic microorganisms. Examples are lactic acid producing bacteria, alcoholic fermentations, oxidation of alcohol to acetic acid, production of other organic acids and amino acids and nucleotides and changes in cellulose, hemicellulose, pectins, gums, fats and proteins.

Lactic acid producting bacteria have been used since time immemorial all over the world in preserving and modifying foods such as cereals (idli, dosai, nan) milk (curds), cheeses and fermented meat and fish.

The lactic acid bacteria convert available carbohydrate to lactic acid where by lowering the pH and changing the conditions suitable for the growth of yeast. Some times propionic acid is also produced which acts as a preservative. They also produce flavour compounds e.g. diacetyl, acetaldehyde and acetoin. This action of lactic acid producing bacteria is observed in the production of many traditional and indigenous foods from fruits and vegetables, legumes, cereals, milk, meat, poultry and fish etc. The acids developed by the organisms during the processing of foods contribute to the flavour of the final product and also act as preservatives preventing the growth of undesirable pathogenic and spoilage microorganisms.

Some of these lactic acid producing bacteria produce lactic acid only or acetic acid, formic acid and ethyl alcohol. The organisms producing only lactic acid are known as *homofermentative* and those producing lactic acid with other compounds are *heterofermentative*. Lactic bacteria normally isolated from vegetable fermentations are: *Lactobacillus plantarum, Lactobacillus brevis, Pediococcus cerevisiae, Leuconostoc mesenteroides* and *Lactococcus lactis.*

10.4 NUTRITIONAL VALUES OF FERMENTED FOODS

It is clear now that early man or our ancestors used to store fruits, vegetables, seeds, cereals and even fish and meat for the lean period when the supply of these commodities were scarce. The first reason was storage in order to check the spoilage. The other reason was to improve the nutritional quality and also palatability and acceptability. Certain flavours such as sweet, sour, alcoholic, meat like are obtained in the final fermented products through microorganisms by fermentation. Soybeans and many legumes are difficult to digest and therefore many fermented products are prepared in the oriental countries e.g., China, Japan, Indonesia and African Countries. Besides flavours, these fermentation also increase the levels of vitamins and also amino acids. In general the important beneficial changes obtained through fermentation are:

- Improvement in the profile of flavours, aromas and textures in food substrates.
- Preservation through lactic acid, alcohol, acetic acid and alkaline fermentations.
- Enrichment with essential amino acids, essential fatty acids and vitamins.
- Detoxification of certain toxic constituents during food fermentations.

10.5 NUTRITIONAL QUALITY OF FERMENTED VEGETABLES AND FRUITS

When we consider this aspect of fermentation, most of the vegetables e.g., cabbage (Sauerkrauts and Korean Kimchi), cucumber, green olives, carrots, onion and various others (tomato, pepper, green peas, cauliflower and mustard leaves) are fermented in European countries. Relatively few vegetables are fermented in India. However, the beneficial effect of fruits and vegetables is as follows:

10.5.1 Beneficial Dietary Effects

As reported by nutritionists and health experts, fermented vegetables are rich in fibre, vitamins and minerals. We all are fully aware regarding the

importance of fibre in our dietary system. Many intestinal and heart problems are associated with less consumption of fibre especially the processed foods. There are several benefits in consuming fermented vegetables with live lactic acid producing bacteria:

Lactic acid bacteria if taken along with fermented vegetables or fruits in the diet lower the blood serum cholesterol level.

These bacteria produce a number of metabolites which are beneficial to human health.

They help in preventing tumor formation in the body due to stimulation of factors responsible for immunity.

Lactic acid producing bacteria inhibit the formation of carcinogenic compounds in the gastrointestinal tract.

They reduce the growth and enzyme production by the intestinal bacteria e.g., enterobacters.

These bacteria and fermented foods if taken as a food, change the microflora by eliminating pathogenic and undesirable organisms in our intestine and colon.

10.5.2 Protection of Vitamin C in Fermented Vegetables

In India, we do not include fermented vegetables in our dietary system. However, in Europe, North America and Korea, cabbage and several other vegetables are fermented and higher levels of vitamin C is found in them due to its synthesis by microorganisms.

10.5.3 Mineral Preservation in Fermented Vegetables

The fermented vegetables in a meal help higher assimilation of iron. Iron bioavailability is better in lactic acid producing bacteria fermented carrots.

10.5.4 Reduction in Nitrate Content

Due to the widespread use of nitrogenous fertilizers, high quantities of nitrates are sometimes found in food products. This nitrate which is harmless as such, is reduced and converted into nitrites which finally gets transformed into highly carcinogenic nitrosamines. During lactic acid fermentation of vegetables, nitrites are converted into NO_2 which goes out as a gas.

10.5.5 Improved digestibility

Many indigestible compounds responsible for gas and flatulence and also sulphur compounds in garlic or onion are broken down to inoccous break down products.

10.6 POSSIBLE HARMFUL EFFECTS

a) A number of amines are formed during the fermentation of cabbage, carrot, pepper and turnip etc., and thus their higher concentrations cause unpleasant flavour to the final product or toxic effect. Ingestion of certain amines can cause headaches, fever and vomiting almost similar to

microbiological food poisoning. Histamine, tyramine, putrescine, and cadaverine are normally noticed in the fermented vegetable products.

b) During the fermentation process D(-)lactic acid is also produced depending on the type and number of lactic acid producing bacteria. D(-) form of lactic acid is not assimilated in the body and thus gets eliminated by the kidney in the form of salts which results in the loss of calcium and magnesium. Normally D(-) lactic acid concentration in the fermented vegetables is low without having any adverse effect on the human health. However, care is needed to restrict the quantity in the meals.

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 why a knowledge of biotechnology is useful in understanding microorganisms in fermentations.

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2. What do you understand by fermentation?

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3. Highlight the importance of harmful and useful microorganisms in the spoilage and improvement of food quality.

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4. List the beneficial changes in food commodities by fermentations.

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5. What are the attractive nutritional characteristics of fermented fruits and vegetables?

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10.7 CLASSIFICATION OF FERMENTED FOODS

It is important to know about different varieties of fermented foods prepared and consumed all over the world depending upon the agricultural and food raw materials produced in that region. These fermented foods can be classified into the following categories:

i) Fruit and Vegetable products

ii) Beverages (alcoholic and non-alcoholic)

iii) Cereal products

iv) Milk Products

v) Fish Products

vi) Products from Legumes

vii) Meat products

viii) Starch Crop products

i) Fruits and Vegetable Products

You are now fully convinced that the fermentation of food commodities was practiced by the early man and by trial and error many technologies were developed. Vegetables have been preserved throughout the world by fermentation. Examples are cabbage (Sauerkraut, Korean Kimchi), radish, mustard leaf, gherkins and cucumbers, ginger onion, chilli and bambooshoots (Malaysian pickles), carrot, turnips and peppers. In India, relatively very few vegetables are fermented and preserved for consumption. Among fruits, olives are commercially fermented and consumed in European countries as an appetizer.

ii) Beverages (alcoholic and non-alcoholic)

Beverages are produced in large quantities in all regions of the world. We normally find two types of beverages which are common everywhere. The first group comprises of alcoholic beverages in which fermentation plays a major role in contributing the flavour and chemical and physical characteristics of the fermented products. Beer and wine fall under this category. After fermentation further distillation is done and thus a variety of products termed 'spirits' such as whisky, gin, brandy, rum etc., are produced.

The second category of beverages are non-alcoholic e.g., coffee, cocoa and tea. All of them involve fermentation. India is a major producer of tea and coffee. If you look at the worlds map, you find an interesting observation

that these are based on the type of agricultural crops cultivated in that particular area depending on the geographical and climatic conditions. The colder countries of Europe including Britain, Scandinavia, Netherlands and Poland consume beer which is manufactured mostly from barley. The southern countries of Europe grow grapes extensively and produce different varieties of wine. These beverages have spread to many countries wherever the European settled e.g. Northern America (The United States of America, Canada), Australia and South Africa. Rice beer in Indian sub-continent, sorghum beer in Africa, sake (rice) in Japan and a variety of alcoholic beverages are produced in different parts of the world. In Europe and North America apples are used for cider production. In warmer climate in Africa, Asia, Oceania (Australia and New Zealand), the Indian subcontinent and South America the sweet liquid sap of palm trees is fermented to wine. In India, it is known as '*Toddy*'. Cashew apple pulp is extensively is used for the preparation of Feni in Goa.

The alcohol content of most of the fermented materials varies between 5-18%. Its concentration is increased in the range of 35-55% by distillation and thus brandy, whisky rum and gin are manufactured. Consumption of excessive amounts of alcoholic beverages leads to intoxification and loss of body control. Therefore it is prohibited in many countries and also many religions of the world.

In contrast to alcoholic beverages, the non-alcoholic beverages most widely consumed throughout the world are coffee, tea and cocoa and these are largely produced in India especially in southern part of the country. The tea leaves are fermented as such by the natural microorganisms. In the case of coffee and cocoa the pulp surrounding the beans are removed by the natural fermentation. This process contributes to the flavour of the final product. Bacteria, yeasts and moulds are involved in the fermentation of these commodities.

iii) Cereal Products

Cereals are the major staple food in every parts of the world. These are wheat, rice and maize. The most popular fermented cereal product is bread which is consumed in every region. It is done by fermenting wheat flour dough with the yeast *Saccharomyces cerevisiae*. Lao-Chao is a fermented rice product of China prepared by natural fermentation containing strains of *Rhizopus oryzae,Rhizopus chinensis* and *Endomycopsis* species etc., Puto of Philippines, Ang-Kak of China, Ragi of Indonesia, Tape-Ketan of Indonesia, Ogi of Nigeria, Injera of Ethopia and Banku of Ghana are produced extensively and consumed regularly in these countries. In India, mixed fermented preparations of rice and pulses and other commodities are idli, dhokla, khaman, papad and jalebies etc., Ambali, bhatura, kulcha and warri are also Indian fermented foods prepared and used in different parts of the country.

iv) Milk Products

Milk products have been included in our diets since time immemorial. In early days, natural fermentation of milk was the best method of preserving milk. Dahi and Chhanchh (butter milk) are important ingredients of every day's meal all over the country. The other products are youghurt, cultured milk acidophilus milk, cheese, Srikhand, Kefir and Kumiss etc.,

Fermented milk products have therapeutic properties along with their nutritional characteristics, wholesomeness and good flavour and digestibility. These qualities are introduced in the product by a number of lactic acid producing bacteria e.g. *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* sub-sp. *cremoris* (cultured butter milk, sourcream, cottage cheese, other soft and hard cheeses), *Lactococcus lactis* sub-sp. *diacetylactis* (sour cream, butter, cheese, butter milk), *Streptococcus thermophilus (*yoghurt*, Lactobacillus delbrueckii* sub-sp. bulgaricus (yoghurt, kefir, kumiss, bulgarian butter milk), *Lactobacillus acidophilus* (Acidophilus butter milk).

Realising the health giving properties of fermented milk products, different varieties of preparations are being marketed all over the world. These are acid alcohol fermented milk products, high acid fermented products, medium acid fermented products, low acid fermented products and whey based beverages.

v) Fish Products

The fermented fish products are popular in some countries. Philippine fish sauce and Vietnamese Nuoc-mam are prepared by fermenting sardines, shrimps and small sea fish etc., Malaysian budu is consumed as a condiment on rice and as a flavouring ingredient in various dishes. Baloa baloa is a fermented rice shrimp mixture of Phillipine and consumed by most of the people.

vi) Legumes Products

Pulses constitute an important component of diet after cereals in Asian countries, especially in India. In India we consume tur or arhar, black gram, green gram, Bengal gram, lentils and a range of beans. Soybeans is also gaining popularity in India. Legumes are rich in proteins, and also in oil and carbohydrates. Unlike cereals, their digestibility is poor and therefore, they are fermented to different products in oriental countries e.g., China, Japan, Indonesia, Malysia, Thailand and several African Countries. Majority of these pulses and beans contain oligosaccharides such as stachyose and verbascose which cause flatus in the intestine. Trypsin inhibitors are also present in these agricultural commodities. Fermentation stimulates these undesirable constituents. Thus the fermentation improves digestibility, nutritional quality and textural characteristics of the fermented products. Tempeh, sufu, soybean milk, soy sauce, natto, bangkrek, khaman, warries and mixed fermented foods containing cereals and pulses e.g., idli, dosai, dhokla etc., are consumed in different regions of the world.

vii) Meat Products

There are not many fermented meat products. These products are dry and semi-dry sausages. In the United States of America, the commercial sausages are Genoa and Salami. Among the popular European brands (dry) are summer sausage, cervelet, thurunger, and Teewurst. Semi-dry-sausages are turkey sausage, fermented frankfurter and Frischwurst etc., In Europe and Western World, fermented sausages are preferred whereas in India, their consumption is almost negligible. The lactic acid producing bacteria e.g., *Lactobacillus plantarum, L. sake, L. curvatus, Pediococcus acidilactici, P. pentosaceus, Lactococcus lactis* are mainly responsible for

fermentation of meat. The safety and shelf life of the products are important because these products are contaminated easily and frequently by pathogenic microorganisms.

viii) Starch Crop Products

Cassava (tapioca) is a major food crop cultivated in several African Countries. It is a staple food to most of the people. It contains cyanogenic glucosides and therefore it must be processed. During fermentation, the cyanide content is reduced completely. Gari, lafun, fufu, peujeum, poi and tape are some of the products. Maize, Sorghum and millets are used for the preparation of fermented products like ogi, uji, koko fube and chika etc.

10.8 GENERAL METHODS OF FERMENTATION

The purpose and importance of food fermentation in our life including food preservation, textural modification and nutritional improvement, has already been highlighted earlier. In recent years, the fermentation industry stands next to information technology (IT) and software industries. It is therefore, pertinent to know general method's used in fermentation for the production of fuels, food and pharmaceutical products and life saving drugs.

There are two distinct types of fermentation which are commonly used for the above-referred purpose. These are:

a) Aerobic fermentation

b) Anaerobic fermentation

Aerobic Fermentation

These are carried out under the aerobic conditions in the presence of oxygen which is required for the growth and product formation by the microorganisms. Majoirty of the fermentations such as antibiotics, single cell protein, enzymes and amino acids come under this category. As listed above, the key of these fermentations is the microorganism/s and raw materials (ingredients) and cultural (pH, temperature, humidity and water content) and nutritional characteristics influence the product formation significantly. In many of the fruit and vegetable and agricultural commodities fermentations, the microorganisms grow at very low moisture level whereas in others contrary to this, it is carried out at very high levels of moisture in the range of 85-90% with adequate supply of oxygen. The former is termed as the solid-state fermentation (SSF) and the latter is called as the submerged culture fermentation.

Solid-State Fermentation (SSF)

SSF refers to the growth of microorganisms on solid materials without the presence of free liquid. It is considered to be economical since low moisture content is used and also does not require expensive equipments e.g, sophisticated fermentors. It is extensively practiced in the oriental food fermentations (miso, tempeh, soysausce, natto), secondary metabolites, enzymes, organic acids and composting etc. Although solid-state fermentation is a simpler and less expensive process of growing microorganisms, recovery of the final product adds finally to the cost during the down stream processing

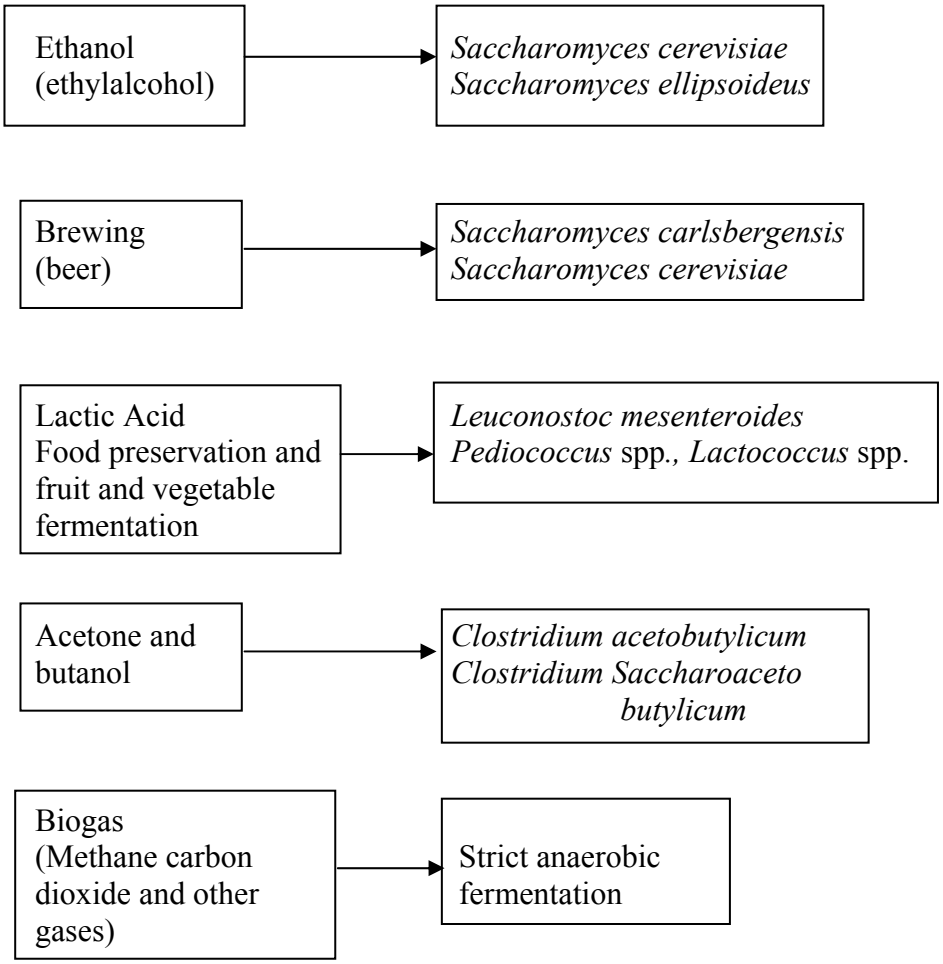
step. However it is widely followed for food and feed enrichment, food and feed enzymes production and composting and waste minimization.

Submerged Culture Fermentation

This method of fermentation has been practised for several years for the production of life saving drugs, enzymes, amino acids and organic acids by bacteria, yeasts, fungi and actinomycetes etc. Oxygen is supplied by either shaking or bubbling air through the liquid medium. Agitation, aeration and temperature affect the fermentation. Batch culture or continuous culture methods are followed for the production of fermented products - commercially.

Anaerobic Fermentation

These are carried out by strict anaerobes or facultative anaerobes such as bacteria and yeasts in the absence of oxygen. Examples of anaerobic fermentation are:



10.9 PRE-REQUISITES FOR INDUSTRIAL FERMENTATIONS

After knowing these preliminary information about the fermentations, it is necessary to know what are the important points to be considered before starting laboratory or industrial scale fermentation.

a) Microorganism/s is the key for any fermentation whereas fermentor is the heart of the process. The choice of a good medium/substrate/raw-material is virtually as important as selecting a right type of strain or microorganism

for the success of fermentation. The medium serves the following purposes:

i) It supplies nutrients for growth.
ii) It supplies nutrients for energy.
iii) It supplies nutrients for building of cell substance.
iv) It is required for the production of final product.

Nutrients needed for the growth and product formation are:

i) Carbon compounds derived mostly from starch, sugar and molasses.
ii) Nitrogeneous compounds.
iii) Inorganic salts.
iv) Water.
v) Vitamins.
vi) Growth factors.
vii) Precursors of fermentation products.
viii) Dissolved oxygen and other gases.
ix) Buffers.
x) Antifoam substances.
xi) Lysate of dead cells.

b) As you have become familiar that fermentation is a microbiological processes hence a potent strain of fungi; yeasts and bacteria is required for desired results. The choice depends upon many factors, the most important being the nature of the raw material. The youghurt preparation requires a strain of *Lactobacillus delbrueckii* sub sp. *bulgaricus* and *Streptococcus salvarius* subsp. *Thermophilus* and milk whereas *Leuconostoc mesenteroides, Lactobacillus, plantarum* and *Pediococcus cerevisiae* become predominant in cabbage for sauerkraut fermentation. In the mixed natural fermentation, it is not possible to control the number and type of microorganisms. Contrary to this in controlled fermentations such as alcoholic beverages, brewing and life saving drugs, an ideal microorganism is needed having the following characteristics:

i) The strain should grow profusely either in liquid or solid state conditions.
ii) The strain should be a pure culture and free of phages.
iii) The strain can be stored for a long period without any genetic change.
iv) The strain should always produce the predictable amounts of metabolite.
v) The strain should be amenable to strain improvement.

c) In nature, microorganisms exist as a mixed culture system and based on the chemical and physical characteristics of the substrate and environmental factors, one or two organisms dominate and thus desired fermentation is achieved. But in monoculture system, these have to be eliminated by sterilization using dry heat, moist heat (autoclaving), radiation and filteration. Sometimes tyndallization and pasteurization are also practiced depending on the nature of substrate and final product. For pharmaceutical

and chemical productions, this step has to be strictly followed as per need of the process.

d) The next step followed is the inoculum preparation and then fermenter has to be inoculated containing cooled sterilized medium at the rate of 1-10%. The fermentation is carried out by controlling all the parameters strictly for a certain period and thereafter the product is obtained by filteration, purification, concentration and drying. The quality of product is monitored at this stage.

10.10 COMPUTER APPLICATIONS IN FERMENTATIONS

The computer application is gaining importance in fermentation industries. It serves two distinct purposes: i) evaluation of fermentation parameters and their impact on the synthesis of desired product in the cell; ii) on line fermentation control especially at the production scale.

Computers find wide acceptability in high valued low volume product formation mainly costly life saving drugs and chemicals. It is used for data acquisition such as information on pH, temperature, viscosity, aeration rate and O_2 and CO_2 content. It also helps in data analysis.

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 important varieties of fermented foods consumed all over the world.

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2. Define ‘Aerobic’ and ‘Anaerobic’ fermentation and cite some examples.

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3. Compare the solid-state fermentation (SSF) and submerged culture fermentation.

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4. What are the requirements of fermentation? Illustrate the significance of microorganism in the fermentation.

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5. Provide an account of computer applications in industrial fermentations.

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10.11 LET US SUM UP

Biotechnology has emerged as an important tool to improve the life of people. It implies the application of microorganisms, plants and animals. Fermentation is an important aspect of biotechnology and it has been in practice since time – immemorial. Both harmful (pathogenic) and useful (beneficial) microorganisms grow on different commodities and environment when physical (water activity, pH, temperature, humidity) and nutritional factors become favourable for their growth. These microorganisms modify the textural and chemical characteristics of food materials and thus make them highly nutritious and palatable. They enrich some of these agricultural food commodities with vitamins, minerals, amino acids and essential fatty acids. These fermented foods are classified into several categories based on the raw materials used for fermentation (fruits and vegetables, cereals, milk and fish and meat products etc.)

The methods of fermentation normally employed are solid state fermentation (SSF) and submerged culture fermentation both aerobic (presence of oxygen and anaerobic (absence of oxygen) conditions. In nature many microorganisms grow together thus mixed culture fermentation takes place whereas in industries one (monoculture) or two microorganisms with improved strains are used and their growth conditions are strictly monitored. Computers are employed in fermentation industries for different purposes.

10.12 KEYWORDS

Fermentation : Fermentation is the chemical transformation of the constituents of raw materials with microorganisms and their enzymes.

Harmful microorganisms : Spoilage and pathogenic microorganisms.

Useful microorganisms : Beneficial microorganisms (wine and beer producing, curd, cheese and bread making and ethanol production).

Homofermentative : The microorganisms only producing lactic acid.

Heterofermentative : Those microorganisms produce lactic acid along with acetic acid, formic acid and ethanol.

Aerobic fermentation : It is carried out in the presence of oxygen.

Anaerobic fermentation : Microorganisms grow in the absence of oxygen (Ethylalcohol, lactic acid, acetone, acetic acid, butanol).

Solid state fermentation (SSF) : Microorganisms grow on solid materials without the presence of free liquid.

Submerged culture fermentation : Microorganisms grows in the liquid medium in the presence of oxygen mostly.

10.13 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   - Emerging area of Science and technology.
   - Application of microorganisms, plants and animals.
   - Production of chemicals, value added products, food materials, medicines etc., commercially.
   - A boon to people all over the world.
   - A myriad of chemicals and products are produced by only microorganisms unlike plants and animals.

2. Your answer should include the following points:

   - Change in chemical and physical characteristics of the raw materials.
   - A process in which innumerable strategic chemicals (ethanol, medicines, harmones, vitamins) are produced by microorganisms.
   - Production of bread, curds, cheese, wine, beer, whisky, rum and brand etc.,

3. Your answer should include the following points:

    - Causing fatal infectious and contagious diseases to human being, animals and plants.
    - Commercially producers of alcoholic beverages of different types from varieties of agricultural commodities, bread, lactic acid fermentation, amino acids, vitamins and antibiotics.

4. Your answer should include the following points:

    - Improves the shelf life of fruit and vegetables.
    - Improves the organoleptic quality.
    - Improves the nutritional quality and health benefits in foods.
    - A good method of storage of agriculture and food commodities.
    - Cheapest and simplest method of preservation.

5. Your answer should include the following points:

    - Fermented fruits and vegetables taken along with lactic acid bacteria lower the cholesterol level in blood.
    - Lowers the number of cardiovascular diseases.
    - Lactic acid producing bacteria present in foods inhibit the formation of carcinogenic compounds.

Check Your Progress Exercise 2

1. Your answer should include the following points:

    - Fruits and vegetables, alcoholic and non-alcoholic beverages, cereals, milk products, fish products, products from pulses, legumes, meat products, starch crop fermented products.

2. Your answer should include the following points:

    - Growth of microorganisms in the presence of oxygen.
    - Growth of microorganisms in the absence of oxygen.
    - Ethanol, beer, acetone, butanol, biogas (aerobic).
    - Enzymes, aminoacids, vitamins and organic acid (anaerobic).

3. Your answer should include the following points:

    - Growth of microorganisms on solid materials for examples grits of cereals, fruits and vegetables, wheat and rice bran etc.,
    - The moisture content is usually maintained around of 32-35% (SSF: Solid state fermentation).
    - Tray fermenters are conventionally used (SSF).
    - Fermentation is carried out in a liquid medium containing 85% moisture (submerged).
    - Sophisticated expensive fermenters along with other equipments are needed (submerged).

4. Your answer should include the following points:

    - Microorganisms are responsible for the conversion of constituents into the desired product.
    - Needs highly potent strains of microorganisms.
    - A good medium for the growth of culture.

- The medium should have optimum quantity of carbon source, nitrogen source, vitamins, minerals, water, pH and oxygen.

5. Your answer should include the following points:

   - Monitoring the functions of fermenters during operation.
   - Data acquisition and data analysis.

10.14 SOME USEFUL BOOKS

1. Steinkraus, K.H. (1995) Hand Book of Indigenous Fermented Foods, Second Edition, Marcel Dekker, New York.

2. Campbell-Platt, G. (1987) Fermented Foods of the World, Butterworths, London.

3. Wood, B.J.B. (1985) Microbiology of Fermented Foods Vol. 1&2, Elsevier Applied Science Publishers.

4. Reed, G. and Nagodawitana, T.W. (1995) Biotechnology, Second Edition, VCH, New York.

UNIT 11 FRUIT AND VEGETABLE-BASED FERMENTATIONS AND THEIR COMMERCIAL PRODUCTS

Structure

11.0 OBJECTIVES

After studying this unit, you should be able to:

- infer the commercial importance of fruit and vegetable based fermentations;
- state the significance of lactic acid fermentation in fruits and vegetables; and
- describe the state of art of sauerkraut, cucumber, kimchi, sinki and fermented pickles production.

11.1 INTRODUCTION

The importance of food fermentation has been highlighted earlier. It is evident that fermented foods are an intricate part of the diet of people in all parts of the world. These are always used as condiments accompanying the main dish. These foods are prepared from plant and animal sources. Fermentation primarily to preserve these commodities and also add flavour and change the texture in order to suit palatability and acceptability. Another important purpose is adding variety to the monotonous diet. Since it has been practiced since time immemorial, it is a house-hold art throughout the world. In recent years, a number of technological and biotechnological developments are taking place and several products are produced on commercial scale.

In early days, people used to collect vegetables and tried to preserve by adding salt. Chinese used to take acid-fermented vegetables during the 3rd century. Korean developed *Kimchi* made from acid fermented cabbage, radish etc., Fermented cabbage is popular in the Western World. Africans evolved the process for acid fermentation of maize, sorghum and tapioca. Even though nothing was known about the technology or microbiology and also about the nutritional quality of the products, the advantages of acid fermented

vegetables, fruits, cereals and milk were well known to people during those days.

During the early twentieth century, it became known that microorganisms are responsible for physico-chemical textural and flavour and taste changes in fermented products. In recent years, some of the fermented foods have been scientifically investigated and based on the microbiological and biochemical information, technologies have been developed for commercial production. Since microorganisms are the key to these fermented food production, these fermented foods can be grouped for convenience as follows:

i) Acid fermentation preserving fruits, vegetables, milk, cereals, fish and meat etc., and enhancing orgnoleptic and nutritional quality.
ii) Protein rich vegetarian foods from legumes and seeds (Tempeh, Oncom etc.).
iii) Alkaline fermentation from beans (Kinema and African fermented foods).
iv) Alcoholic foods and beverages (Ethanol is a major product).
v) Sauces, pastes etc.

Microorganisms grow on the substrate and based on the chemical nature of the substrate, they bring about changes. Two types of acids are predominantly produced viz. lactic acid and acetic acid by microorganisms and thus a variety of products from agricultural commodities are produced especially from milk, cereals, and fruits and vegetables. The other important group of fermented foods is the protein enrichment and modification mainly from soybeans, peanuts and other legumes and pulses. Among beverages, alcoholic fermented beverages e.g., wine, beer top the list all over the world.

In recent years, vegetarian foods are considered to be ideal for human health. Among these lactic acid producing bacteria fermented foods are the best as these organisms tend to help in avoiding cardiovascular and heart diseases, cancer and several other gastrointestinal problems. The advantages of acid food fermentation are:

i) They avoid spoilage of foods and mould and bacterial toxins production.
ii) They preserve the food and assure the foods safety.
iii) They modify the flavour of the original ingredients and improve the nutritional value.

Since canned and frozen foods are beyond the reach of majority of people especially in rural areas, acid fermentation of agricultural commodities remains one of the most simplest and inexpensive method. However, in India acid fermentation of vegetables and fruits are not common as compared to European, Oriental and African countries. The raw materials containing high sugar content favour usually ethanol generation and its conversion to acetic acid. The fruits and vegetables having low level of sugar, allow lactic acid producing bacteria to proliferate which lowers the pH around4.0 due to lactic acid production.

11.2 LACTIC ACID FERMENTED FRUITS AND VEGETABLES

Fermented vegetables are common ingredient of European diet. Sauerkraut, olives and cucumbers are often fermented. The method of vegetable fermentation has been standardized over the centuries both for natural (spontaneous) or addition of starter cultures. The lactic acid producing bacteria grow on the substrate (vegetables) and convert sugar into acids (lactic and acetic). The gradual disappearance of carbohydrate (carbon content) leads to the production of lactic acid which ensures food stability. The pH about 4 of the fermented products inhibits the growth of spoilage microflora and pathogenic organisms.

Salt is added in good quantity which promotes the growth of lactic acid producing bacteria and checks proliferation of contaminating and spoilage microorganisms.

Fruits and Vegetables suitable for Lactic Fermentation

- Cabbage, cauliflower, broccolys, mustard
- Carrots, turnips, beetroots, radish
- Cucumber, Olives, tomatoes, peppers, green-beans and green peas
- Onion and garlic
- Apples, pears, green mangoes, banana, lemon, lime

11.3 SAUERKRAUT (CABBAGE) FERMENTATION

Sauerkraut or Sauerkohl is a German term which means *'Sour Cabbage'* Sauerkraut is extensively used in the North America (Canada and U.S.A.), Germany, Holland, France, U.K. and other European countries. Cabbage (‘*Brassica oleracea*) normally grown in cold climate is found to be suitable for the fermentation purpose.

i) Processing

Fresh cabbage is taken, cleaned, trimmed and shredded into 2-5mm size and finally filled into wooden vats or cement tanks. Salt is added at the rate 2.25% and mixed thoroughly. The top portion of the vat or tank is covered with plastic and enough weight is applied in order to make it compact and allow anaerobic conditions prevail for fermentation. When weight is applied, the salt dissolves in the sap which is expressed by the pressure and by osmosis it comes out from the cells. The leaves respire for sometime and oxygen is utilized and thus anaerobic conditions are created. The leaves shrink in size due to water removal. A spontaneous lactic acid fermentation follows. Fermentation is done for about 30 days or more until 1% lactic acid is formed. The Sauerkraut is removed from the vat and packed in cans, glass or plastic containers. In cans the fermented product is pasteurized at $74^{o}C$ for 3 minutes. Sodium benzoate or potassium metabisulphite is added when product is packed unpasteurized. It is stored at $\pm 5^{o}C$.

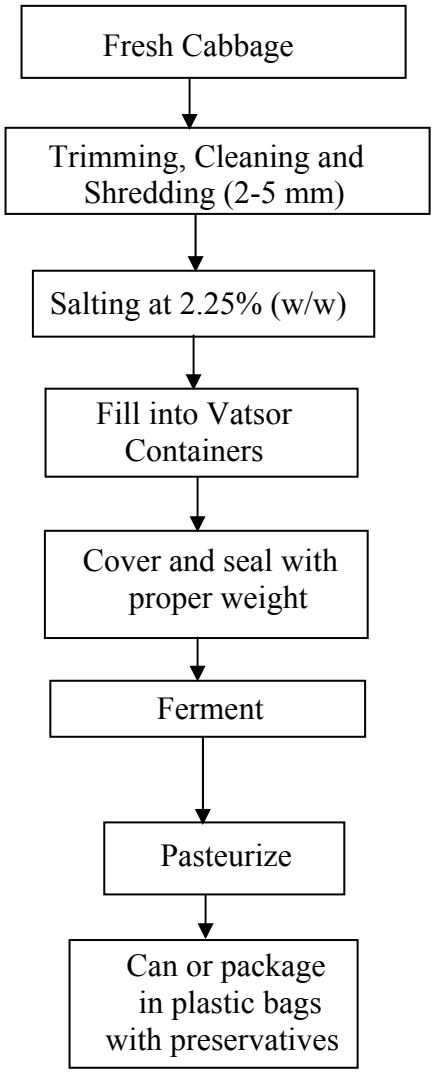

Figure 11.1: Sauerkraut (Cabbage) Fermentation

ii) Microorganisms Involved in Fermentation

Initially the shredded and processed cabbage leaves contain very high number of aerobic bacteria with low counts of lactic acid bacteria. During fermentation, the number of lactic acid bacteria increases suppressing the growth of undesirable organisms and a distinctive flavour of sauerkraut develops with about 1% of lactic acid content. The microorganisms follow usually the following pattern in succession: *Streptococcus faecalis, Leuconostoc mesenteroides, Lactobacillus brevis, Pediococcus cerevisiae, Lactobacillus plantatrum.* Initially heterofermentative nominate the numbers which is taken over by the homofermentative type of microorganisms. Sometimes a previous batch brine is added as a starter culture to facilitate quick fermentation.

iii) Spoilage

Sometimes fermented products are very badly spoiled by contaminating bacteria causing off flavours and colour and undesirable texture. Temperature, salt concentration and sanitary conditions are important to control the desired fermentation. Lower temperature around 7-10^{o}C

favours slow growth of bacteria and thus allows good fermentation. In traditional system, fermentation is allowed for 6 months.

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. List some of fruits and vegetables used commonly for fermentation and the mode of lactic acid production by the lactic acid producing bacteria.

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2. Define the word sauerkraut.

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3. Explain, how sauerkraut is prepared.

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4. Cite some microorganisms involved in cabbage (kraut) fermentation.

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5. What are the causes of spoilage of sauerkraut?

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11.4 CUCUMBERS FERMENTATION

Cucumbers (*Cucumis sativus)* are grown throughout the world. It is reported to have been originated in India. A number of varieties of cucumbers are cultivated in fields or greenhouses for table or pickling purposes.

i) Processing

Cucumbers are washed after selecting them and placed in a covered tank containing a salted and acidified brine. The brine is normally acidified (vinegar or acetic acid) to pH 4.5.The salt concentration of the brine is maintained between 5-8%. Too little salt less than 5% favours the growth of enterobacteriaceae bacteria. Higher levels of salt allows growth of yeasts. After the initiation of fermentation, almost everyday the brine is buffered with sodium acetate. During fermentation, contamination with yeasts is observed and therefore, to avoid bloater formation, the brine is purged with nitrogen or air. Potassium sorbate (0.035%) is also added to check the growth of yeasts and moulds preferably if fermentation is done at smaller scale. Fermentation if it is carried out at 15-20^{o}C, it takes normally 3 weeks to 1 month.

ii) Microorganisms involved in the fermentation

Streptococcus faccalis, Streptococcus lactis, Leuconostoc mesenteroides, Pediococcus pentosaceus, Lactobacillus brevis, Lactobacillus plantarum, Lactobacillus bavaricus are usually noticed during the fermentation. Controlled fermentation and pure culture fermentation have been studied but not commercially exploited. Glucose and fructose are converted to lactic acid, acetic acid, ethanol, mannitol and carbon dioxide. About 1.1%, lactic acid is present after the fermentation. Textural properties after fermentation is important. A firm and crisp texture is desired. A clean flavour is also desired.

iii) Spoilage

Bloater formation is observed due to the growth of gas forming microorganisms e.g. yeasts, bacteria and other contaminating organisms. Bleaching of the green colour of cucumbers takes place due to exposure of fruits to sunlight. Offensive flavours are produced due to the formation of butyric and propionic acids.

11.5 KIMCHI FERMENTATION

Kimchi is a major condiment of Korean diet. It is a popular dish served at every meal along with cooked rice. Kimchi is a fermented product, gaining popularity in the United States of America and other countries. It is made from cabbage and other vegetables like radish, mustard greens, cucumbers, green onions, Chinese leeks, turnips and green peppers, spinach, pumpkins and egg plants.

i) Processing

Good quality cabbages are selected, cleaned and cut into smaller pieces. The cut cabbage is placed in a brome solution containing 5-7% salt concentration for 12 hours. The brined vegetables are rinsed and drained. Seasoning ingredients are thoroughly mixed and filled in earthen jars or glass jars. The jars are buried (80-90% of container depth) underground. These are covered on the top with bundles of rice straw to protect from sunlight and rain. This technique is followed in the rural areas. In urban areas the mouth of the jar is covered with a cloth piece and kept in shaded places. Low temperature is considered to be ideal for slow fermentation. Usually less than 15^{o}C temperature is optimum for good quality Kimchi.

ii) Microorgansims involved in the fermentation

The microorganisms involved in Kimchi fermentation are *Leuconostoc mesenteroides, Streptococcus faecalis, Lactobacillus brevis, Lactobacillus plantarum, Pediococus cerevisiae.* The aerobic bacteria found are species of *Achromabacter, Flavobacterium* and *Pseudomonas.* The main bacterium responsible for Kimchi fermentation is *Leuconostoc misenteroides.* The fermentation is initiated by *Leuconostoc mesenteroides* and terminated by *Lactobacillus plantarum / Lactobacillus brevis.*

The organic acids produced during fermentation contribute to the final flavour of Kimchi. The organic acids normally noticed in Kimchi are citric, fumaric, oxalic, malonic, malic and succinic acids. Freshly fermented, good quality Kimchi should have distinct red or green colour with pleasant flavour and taste.

iii) Spoilage

Softening is the major problem in Kimchi fermentation. Yeasty and off flavours also effect the quality of the fermented product.

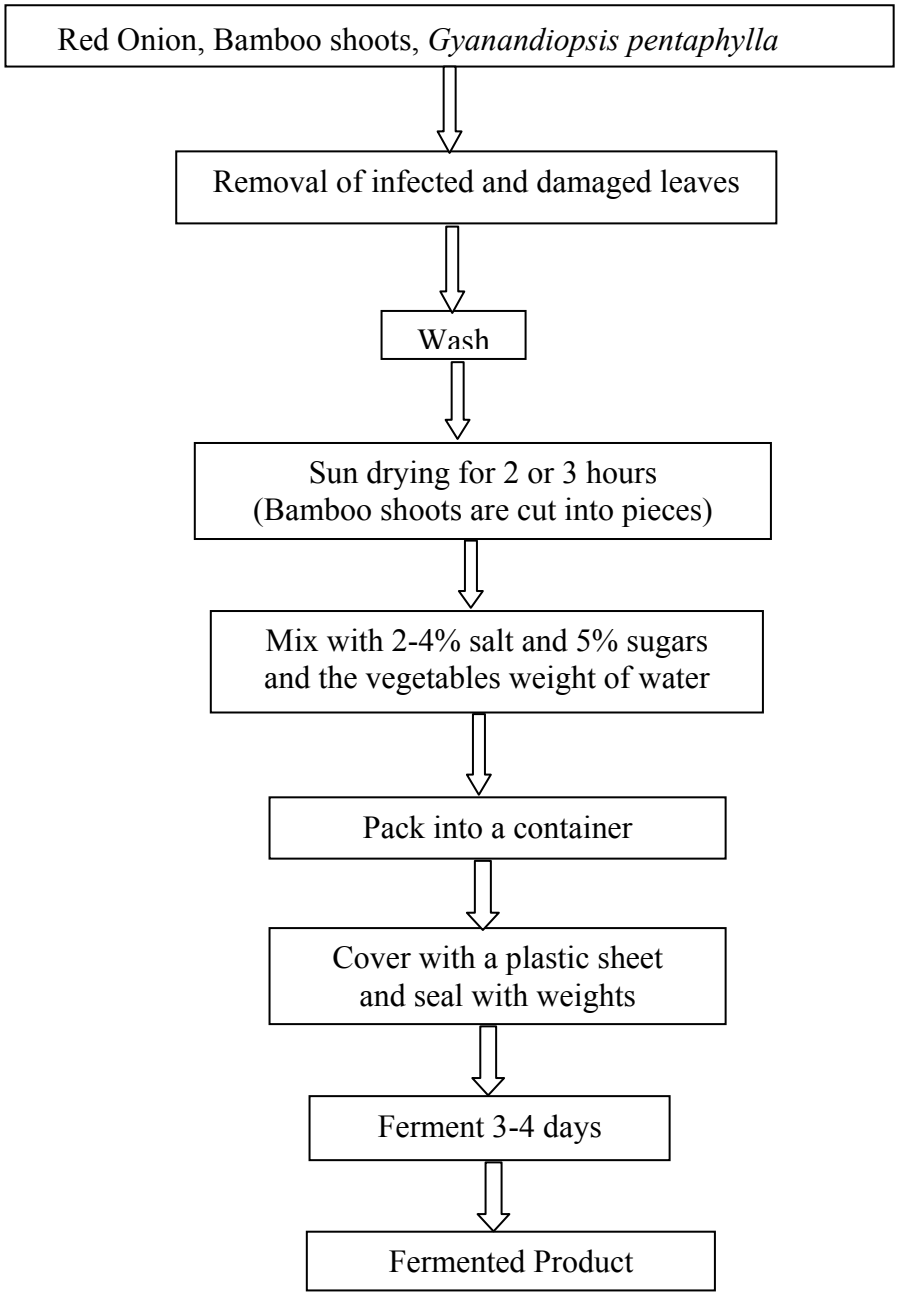
11.6 INDIAN SINKI FERMENTATION

Sinki is consumed as a pickle in the Himalayan belt of India, Nepal and Bhutan. It is prepared from the tap root of radish. Fresh radish roots are washed and placed outside for sun drying for 1 or 2 days. These are shredded and washed again and packed tightly into an earthen ware or glass jars, sealed and left for fermentation. Fermentation is carried out for 15-30 days at room temperature around 30^{o}C. Initially *Lactobacillus fermentatum* grows followed by *Lactobacillus brevis* and *Lactobacillus plantarum.* The pH drops to3.3 and acidity rises to 1.28%. When fermentation is over, the fermented radish is sundried for 3-5 days. It is fried with salt, tomato and green chilli. The fried mixture is then boiled in rice water and served hot as soup along with the main meal.

11.7 FERMENTED PICKLES

i) Pak-Sian-dong and Related Fermentations (Fermented bamboo shoots and fermented red onion)

These are the common pickles of Thailand. The fresh vegetable is washed thoroughly and spread on a mat in the air or sunlight in order to loose water and tissues to wilt. It is mixed with 2% salt, 4% sugar and the same quantity of water and kept in a tight container. It becomes ready for consumption in 3-4 days. Acidity normally reaches to 0.8% with pH around 3.9-4.0.



Microorganisms involved : *Leuconostoc mesenteroides, Pediococcus cerevisiae,*
Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus fermentii.

Figure 11.2: Pickles (fermented) of Thailand

ii) Malaysian Pickles

Home made pickles are common in most of these countries especially in rural areas. Fruits and vegetables which are relatively sour, are used for pickle preparation. Many types of fruits and vegetables are used depending upon the availability and seasonality. The common vegetables used are: gherkins, cucumbers, ginger, onion, leek, chilli, bamboo shoots, unripe

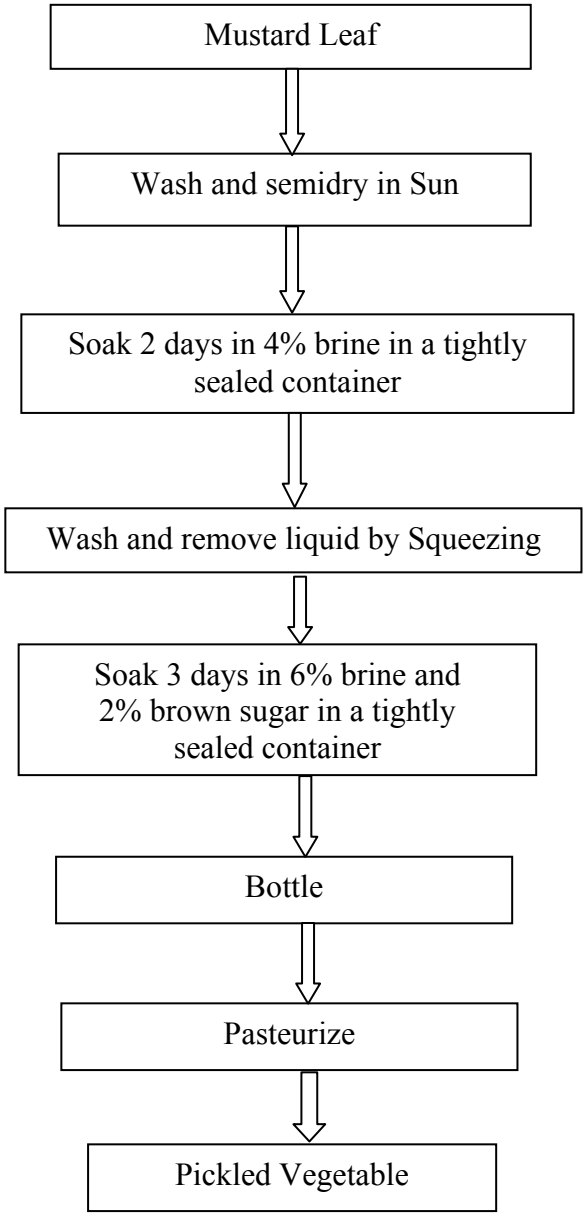


Figure 11.3: Malaysian pickled vegetable

mango, papaya, lime, lemon and nutmeg. In all these fermentations higher level of crystal salt or brine is used in curing the tissues. Sugar and other fermentable carbohydrates, dilute vinegar, and spices are also added. It appears to be a simple process but the quality differs from house to house. Pickles undergo a bacterial lactic acid fermentation. The same group of microorganisms are involved in the fermentation. Fermentation takes about 8 days depending upon the substrate.

In India, several varieties of pickles are prepared both for indigenous consumption and export purposes. We usually prefer non-fermented pickles preserved in edible oils. The quality and taste differ based on the ingredients used. Bamboo shoots pickles, brinjal and mixed vegetable pickles are common in North India. Some of these are exotic in nature prepared at house-hold level.

iii) Lactic Fermented Fruits in European Countries

Fruits are cleaned and blanched at 50-60^{o}C to destroy undesirable bacteria. After the addition of *Lactobacillus acidophilus* or *Lactobacillus bifidus* the product undergoes to lactic acid fermentation. The fruit is shredded and pulverized and pasteurized and finally goes for marketing.

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. Highlight the importance of cucumber fermentation and different steps involved in fermentation.

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2. Name the countries where Kimchi is prepared and used. List vegetables used in Kimchi fermentation.

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3. Explain about the Indian sinki fermentation and method of its preparation.

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4. Give a brief account of fermented bamboo shoots and fermented red onion.

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11.8 LET US SUM UP

Fermentation of different types of agricultural commodities has been practiced with an aim to preserve them and also add exotic flavour and change the texture. Fermented fruits and vegetables have been in use mostly as condiments in order to avoid monotonous diets. Lactic acid producing bacteria grow luxuriantly in favourable conditions on fruits and vegetables and produce good quantity of lactic acid and other acids and antimicrobial substances which lower down the pH and thus check the growth of spoilage and pathogenic microorganisms. It is a simplest method of preservation. Sauerkraut and cucumber fermentations are the typical examples. Kimchi of Korea, Sinki of Indian sub-continent, fermented bamboo shoots and red onion and Malaysian pickles are produced commercially and consumed by the people. Lactic fermented fruits are becoming popular in European countries.

11.9 KEYWORDS

Sauerkraut	**:**	Fermented cabbage.
Kimchi	**:**	Korean fermented product (cabbage).
Sinki	**:**	Indian fermented product from radish.
Pak-Sian-dong	**:**	Fermented bamboo shoots of Thailand

11.10 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   - Cabbage, cauliflower, broccoli, mustard, carrots, turnips, beetroots, radish, cucumber, olives, tomatoes, peppers, green beans, peas, onion, garlic, apple, pears, green managoes, banana, lemon and lime.
   - Lactic acid producing bacteria grow on vegetables and fruits and convert sugars into acids (lactic and acetic).

2. Your answer should include the following points:

   - A German term meaning ‘Sour Cabbage’.

3. Your answer should include the following points:

   - Fresh cabbage → trimming, cleaning and shredding → salting → filling into vats → covering the vat and applying weight on the top → allowing fermentation → pasteurization → packing with preservatives.

4. Your answer should include the following points:

   - *Streptococcus faecalis, Leuconostoc mesenteroides, Lactobacillus brevis, Pediococcus cerevisiae.*

5. Your answer should include the following points:

   - Good sanitary conditions needed while processing cabbage for fermentation.
   - Low temperature and appropriate salt concentrations favour good fermentation and the final product.

Check Your Progress Exercise 2

1. Your answer should include the following points:

   - Widely cultivated in India in all the States irrespective of climatic changes Selection of fruits → washing → placing fruits in brine 5-8% (pH 4.5 adjusted with vinegar) → allowing natural fermentation at 15^{o}C → purging nitrogen to avoid bloater formation → fermentation for one month.
   - *Streptococcus faecalis, Streptococcus lactis, Leuconostoc mesenteroides, Lactobacillus brevis, Lactobacillus plantarum*.

2. Your answer should include the following points:

   - A major condiment of Korean diet.
   - Vegetables like cabbage, radish, mustard (green), cucumbers, green onions, Chinese leeks, turnips, green peppers, pumpkins, etc.

3. Your answer should include the following points:

   - Radish roots → sundrying for 1-2 days → shredding → packing tightly in an earthen vessel → sealing of the mouth of vessel → allowing fermentation for 15-30 days → fermented radish sundried → used as a condiment for soup.

4. Your answer should include the following points:

   - Fermented vegetables of Thailand.
   - Fresh vegetables washed and spread on a mat and sundried.
   - After sundrying 2% salt and 4% sugar are added and mixed with the vegetables quantity of water and placed in a container.
   - Fermentation normally takes 3-4 days.

11.11 SOME USEFUL BOOKS

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UNIT 12 FRUIT-BASED ALCOHOLIC BEVERAGES

Structure

12.0 OBJECTIVES

After studying this unit, you should be able to:

- discuss about different varieties of wine produced commercially in different parts of the world;
- know the microorganisms involved in wine fermentation and factors influencing its quality;
- explain the method of wine production and different practices involved before and after fermentation; and
- get information on clarification of fermented juice and maturation of wine.

12.1 INTRODUCTION

Production and consumption of fermented beverages is an old practice. It has been referred in Vedas. In the previous chapter, how lactic acid from plant constituents is produced to preserve the food along with favourable organoleptic changes are discussed. Similarly many fruits contain very high amounts of free sugars which are readily converted into ethanol and carbon dioxide with the help of yeasts and thus alcoholic beverages are produced.

Wine is normally referred as a by-product of grapes (*Vitis vinifera)*. However, wine can be produced from any fruit or flower rich in soluble sugars. Still most of the wines are produced from grapes throughout the world. European, Western World, North America especially colder climate countries are the major producers and consumers of alcoholic beverages. In these countries, 75% of the crop is diverted for wine production. France and Italy are the major producers of quality wine. Due to several reasons including the taboo attached

to consumption of alcoholic beverages, fruit wine and brandy industries have not developed well in India. We meet most of our demand through import although India is the second largest producer of fruits and vegetables.

12.2 TYPES OF WINE

As stated already, the pressed juice of grapes or any fruit rich in soluble sugars if fermented by yeast/s or spontaneous natural fermentation, it results in the ethanol (wine) production and its disstilate is known as "Brandy". White wine is produced from the grapes usually cultivated whereas the red variety or Bangalore blue grapes having purplish red skin give rise to "Red Wine".

Different types of commercial wines are listed below:

- *Champagne* is a foaming wine which contains dissolved carbondixoide.
- *Sparkling* wine is produced by carbonation by injecting carbondixoide into the wine.
- *Liqueur Wine* is sweet with a higher content of alcohol.
- *Sherry* is a fortified wine with two distinct styles, the first being dry wine (without any sweet taste) consumed as an appetizer before meals and the other being olorosos sweetened wine taken after meals.
- *Port* in which wine is stored in a wooden cask or Hintage ports. During maturation the type of barrel used for aging contributes to quality of wine.
- *Vermouth* is a flavoured wine in which different varieties of herbs and spices are added.
- *Brandy*: It is a distilled wine stored mostly in wooden cooperage or casks.

12.3 FRUITS USED FOR WINE-MAKING

Sugars present in fruits are responsible for quick fermentation and other constituents contribute to the flavour and aroma of the wine. Grapes, apple, pear, custard-apple, mango, jamun, coconut sap, palm sap, pomegranate, banana, guava, ber, plum, orange, litchi, dates, pineapple, strawberry, raspberry, cherry, grape - fruit etc. can be used for wine making. The technology for grape wine has been developed in the western world and thus quality products are produced commercially. There are many reasons for non-acceptability of non-grape fruits for commercialization. Many of these fruits contain low levels of soluble sugars and their fermentability is poor. Extraction of juice from these fruits poses serious difficulties. Some of these have very high or low concentrations of acidity which influences the quality of wine considerably. Bitterness and some other organoleptic defects of the final product are the other serious problems for commercialization.

12.4 IMPORTANT FACTORS INFLUENCING THE QUALITY OF WINE

i) Fruits and their chemical composition

Grapes are the best fruit for quality wine production. It has been found that the cultivation conditions of grapes influence the quality of wine. Fruit variety, sugar content, additives, pulp or juice yield, yeast strain for fermentation, filteration, maturation and preservation of wine, all affect the final quality of wine. Yeast is the key for alcoholic fermentation or ethanol production and the factors which influence the growth of yeast also affect the quality of the wine. The sugar content of selected fruits is given in Table 12.1.

Table 12.1: Sugar and acids composition of fruits

Fruit	Total Sugar (% Fresh Weight)	Acid (% of Tartaric acid)
Apple	8-10	0.9 - 1.4
Banana	18	0.3 - 0.4
Grape	15 - 22	0.4 - 1.3
Orange	9 - 10	0.8 - 1.1
Pear	9 - 11	0.2 - 0.4
Strawberry	5 - 6	0.6 - 1.5

ii) Temperature

The temperature of must (fruit juice) strongly influence the yeast growth. The yeast normally used for wine fermentation is strain of *Saccharomyces cerevisiae*. The optimum temperature for wine fermentation ranges between 22-27^{o}C. Quality white wine is produced at lower temperature 10-15^{o}C where as red wine needs higher temperature which ranges between 20-30^{o}C.

iii) Ethanol Content

As we know one mole of glucose gives rise to two moles of ethyl alcohol (ethanol) and two moles of carbon dioxide during the fermentation.

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2$$

Beyond certain concentrations of sugar in the must/mashes, the yeast growth is stopped. Similarly after 10-12% (v/w) ethanol in the must, the growth of yeast declines considerably. There are a number of methods known to enhance the ethanol tolerance of the selected strain used for fermentation.

iv) Carbohydrate and Energy Sources

It is apt to think that the wine is produced from the sugar and flavour containing chemicals present in the fruits with the help of yeast/s. It is, therefore, important to know the type of sugars and their quantity present

in the must. Glucose, fructose, sucrose and maltose are easily utilized by the yeast (*Saccharomyces cerevisiae*) for its growth and ethanol and carbon dioxide production. Lactose, pentoses, dextrin, starch or higher molecular weight carbohydrates or poly-saccharides are not utilized by the yeast. Usually 0.8-1.3% of sugar is utilized for the growth and rest is used for ethanol generation. At sugar concentration of 25% and above in the must, the yeast growth is slowed down. Except grapes, due to low concentration of sugars in other fruits, the mash has to be supplemented with sugars.

v) Carbon dioxide and Pressure

Carbon dioxide is produced during the fermentation and if higher pressure is built then it inhibits the growth of yeast. However, the sparkling wine is produced under the carbon dixoide pressure in a closed container.

vi) Sulphur Dioxide (SO_2) and other Agents

Sulphur dioxide is added to the must or juice in order to bring down the number of contaminating microorganisms viz., moulds, yeasts and bacteria. The other chemicals tried are salicylic acid, bromoacetic acid, ethylene oxide, benzoic acid and sorbic acid. SO_2 is preferred because it is less toxic to human beings.

vii) pH and Acids

Normally the organic acids present in fruits do not interfere with yeast fermentation. Other organic acids such as acetic, butyric, propionic and fatty acids have inhibitory effect on yeasts. If the pH is below 3.0, the alcoholic fermentation is delayed considerably.

viii) Growth Factors

Biotin, inositol, nicotinic acid, pantothenic acid, p-aminobenzoic acid, pyridoxine and thiamine are needed for the growth of yeast.

ix) Minerals and Pesticides

Iron, copper, zinc and aluminium are present in the final product. Fermentation is not usually affected by the presence of lower concentrations of these metals. They normally come through presses or other equipments used. Pesticides are normally used to control pests which infest trees and fruits. Their residues in the wine is highly objectionable.

x) Nitrogen Sources

For the growth of yeast, nitrogenous compounds are needed. The amino nitrogen present in the must supports the growth of yeast. There are many factors e.g. method of juice preparation, ripening of the fruit, variety and several other cultivation parameters, which influence the amounts of these chemicals. Some of the fruits are deficient in them and therefore, supplementation is necessary. The inorganic nitrogenous salts usually used in wineries are: $(NH_4)_2\ HPO_4$ or $(NH_4)2SO_4$ upto 0.3 g/l.

xi) Tannin/Phenolic Compounds

Tannin and phenolic compounds are present in the fruit juice. Their concentrations differ from fruit to fruit. Tannin normally does not affect the growth of *Saccharomyces cerevisiae* (yeast).

xii) Juice Clarification

In recent years, grape and apple juices are being treated with pectinolytic enzymes for better yield and quality of wine. However, in wine manufacture, cloudy or turbid juice is preferred. Bentonite is added to improve the fermentation. However, the fruit pulp of non-grape fruits have to be treated with pectinolytic enzymes. There are many reports that musts which contain insoluble particles ferment quickly. Therefore, insoluble materials such as filter aids or wheatflour is added in the musts.

12.5 MICROORGANISMS INVOLVED IN WINE-MAKING

i) Yeasts

The quality of wine depends on the yeast strain used for fermentation. The quick growth of yeast and an efficient conversion of sugar into ethanol decide mostly the quality of wine. *Saccharomyces cerevisiae* is the most important yeast for the juice (must) fermentation. Besides this yeast, there are numerous other species of yeasts which are also present and some times they spoil the wine.

Saccharomyces cerevisiae and numerous other yeasts such as *Kloeckera apiculata, Hansenula anomala, Candida stellata, Candida krusei* are also present in grapes. Besides these, *Schizosaccharomyces pombe, Saccharomyces bayanus, Saccharomyces fermentii* have also been isolated from grapes and various other fruits.

When grapes are crushed, within a few days *Saccharomyces cerevisiae* grows and multiplies to a higher number of cells. In the case of spontaneous fermentation, inoculum is not added and the yeasts present on the substrate and equipments serve as a source of starter culture. In order to maintain the quality and yield of wine, wineries all over the world are using a particular standard strain of *Saccharomyces cerevisiae.* It also provides protection from the 'Killer yeast'. Killer yeasts are infected with a virus that destroys the used strain of yeast for fermentation. Some times an active dry yeast culture about 0.1 g/L is also added to accelerate the fermentation.

It is an important question why wineries prefer *Saccharomyces cerevisiae?* It is tolerant to high levels of sugar of fruit juices, tolerates high ethanol concentration, invariably grows even at low pH, ferments must at low temperature and resistant to sulphur dioxide level which suppresses the growth of bacteria, yeasts and moulds. Apart from these, it induces desirable aroma in the wine. That is the reason it is called as wine yeast.

Morphology of *Saccharomyces cerevisiae*

It is spherical to ellipsoidal in shape with 8 × 7μ of size depending on the growth medium. It multiplies by budding.

Most strains of *Saccharomyces cerevisiae* are capable of producing alcohol upto 16%.

ii) Microorganisms other than yeasts in Winemaking

Bacteria

Lactic Acid Bacteria
Leuconostsoc oenos
Pediococcus pentosan, Pediococcus purvislus, Lactobacillus plantarum
Lactobacillus fermentum
} Responsible for malo-lactic fermentation and spoilage

Acetic Acid Bacteria

Acetobacter and *Glucanobacter spp.* } Responsible for Vinegar taste, spoilage and stuck fermentation.

Bacillus and *Clastridium* spp. } Spoilage

Fungi

Botrytis cinerea
Penicillium sp.
Aspergillus sp.
} Botrytized wine, spoilage, corkytaints

Actinomycetes
Actinomyes spp.
Streptomyces spp.
} Earthy and corky taints.

12.6 PREFERMENTATIVE PRACTICES IN WINE-MAKING

i) Stemming, Crushing and Juice Separation

Fruit stalks, seeds and skins (pomace) are removed and then fruits are crushed and macerated. To check initiation of fermentation, white grape juice is chilled to about 10^{o}C. Sulphur dioxide is also added to retard the multiplication of microorganisms. Red grapes are given longer time for maceration at 24-27^{o}C which helps the extraction of pigments and phenolic compounds. Pressing separates the juice from the seeds and skins of the fruit. Large wineries use dejuicers for this purpose.

ii) Clarification

If juice is allowed for some hours for settling, the solid materials and suspended particles get separated and a clear juice is obtained. However, in commercial wineries filter aids such as bentonite is used and juice is centrifuged at low speed or passed through filter press.

iii) Juice/Must Adjustment

Total acidity of must is important for the final quality of wine. If acidity is less than the desired concentration, citric or tartaric acid is added appropriately. Higher level of acidity is retarded by the supplementation of calcium or potassium salts. Sugar is added in the must when sugar content is too low in the fruits. It is known as chaptalization in wine making. The other techniques followed for increasing sugar concentration are reverse-osmosis, cryoextraction and entropie concentration.

12.7 FERMENTATION

Fermentation is an energy releasing metabolism in which the electron donor and electron acceptor are organic compounds. In wine fermentation, glucose and fructose are the electron donors and ethanol is the acceptor. Glycerol, acetic acid, acetaldehyde and succinic acid are also electron acceptors which are produced in the must along with traces of diacetyl and fusel (higher alcohols).

Fermentation is done in a vat (open top) or tanks (sealed top). As stated earlier, both spontaneous and starter (inoculum) addition fermentation is practiced for wine making. During the initial stages, aerobic yeasts *Hansenula anomala* and *Klockera apiculata* are active. Subsequently they are taken over by the *Saccharomyes cerevisiae. S. cerevisiae* multiplies at a fast rate within 3 to 4 days and then ethanol production follows rising its level gradually and steadily.

12.8 SPOILAGE OF FERMENTATION AND WINE

Due to over clarification of juice/must and the presence of high number of wild yeasts having killer viruses and some other reasons some times fermentation does not proceed well. It is referred as the 'Stuck Fermentation'. Several species of lactic acid bacteria e.g. *Leuconostoc oenos, Lactobacillus* and *Pediococcus* spp. grow in the must and convert malic acid into lactic acid. Malic and tartaric acids are the major constituents of grape juice. They spoil the taste of wine. The wine and cider are spoiled by the acetic acid producing bacteria. They grow on the surface and oxidize ethanol to acetic acid. Similarly wild yeasts grow on the surface of stored wine and spoil the product. The sugar present in wine is converted into lactic acid by several spoilage microorganisms. Butyric acid bacteria, slime producing bacteria and moulds spoil the final stored product. Changes in colour of wine take place due to oxidation. It is, therefore, necessary to take care while carrying out the fermentation and during the maturation and storage of wine.

12.9 POST-FERMENTATION PRACTICES

i) Clarification and Stabilisation

Clarification refers to methods adopted for clear wine for bottling while stabilization assures that wine remains clear after bottling. Fermented must is allowed to settle for clarification. Dying yeast cells, grape cell remains, precipitated tannins and crystallized salts are removed. Clarification of wine is sometimes achieved by centrifugation.

ii) Fining

To remove colloidal materials, fining is practiced using filter aids bentonite, kiesselghur, silicondioxide, gelatin, albumin and isinglass.

iii) Centrifugation

It is done to remove fine particles.

iv) Filtration

Sometimes filtration is done with a courser filter but membrane filters are used to remove fine particles and live cells of microorganisms.

v) Crystallization

Crystallization of potassium and calcium tartrate salts occurs in wines. It has to be removed by the cold filtration adding salt at low temperature ($-5^{o}C$). Crystallization is undesirable for expensive wines.

vi) Haze formation

Normally soluble proteins and tannins precipitate in the must and lead to haze formation. The judicious addition of pectinase, β-glucanase or mixture of Kisselghur and gelatin prevents the haze formation.

vii) Adjustment of Factors

Adjustment of acidity, pH, sweetness, alcohol content and decolouration of wines are practiced based on the requirement of the product in the market. Blending is done all over the world in order to get the desired fragrance.

viii) Maturation

Freshly prepared wine is always found to be harsh with yeasty flavour. Maturation develops the mellowed wine taste with fruity and desired flavour. The quality of wines depend on the maturation and ageing. The maturation period extends upto 6 to 12 months. There are several methods used for maturation. The oldest and traditional method is to allow maturation of wine in the same barrel in which fermentation occurred. The other method is to employ oak wood barrels which results in the best quality of wine. Long oak strips or chips are dipped in the filtered wine and

maturation is allowed to take place for more than a 6 months. Several chemical changes take place during this process.

Bottling

Dark amber colour glass bottles with a distinctive neck are commercially used. Usually oak cork are used as a closure conventionally.

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 wine and give examples of different varieties of wine used all over the world.

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2. Explain, how wine is produced from fruits.

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3. What is 'MUST', name some yeasts involved in wine fermentation?

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4. What does the 'Killer yeast' indicate?

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5. Why does winery prefer *Saccharomyces cerevisiae?*

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6. What are prefermentative practices in wine making?

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7. What do you understand by the 'Stuck fermentation', Provide information about post-fermentation practices?

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12.10 WINE FROM DIFFERENT VARIETIES OF FRUITS

i) Apples

Cider and wine is produced from apple. Cider is a low alcoholic drink, contains alcohol between 4 to 6%. The apple varieties should have moderate amounts of phenolics. The higher juice containing varieties are preferred for cider fermentation. Apple juice or concentrate is used for wine fermentation. The alcohol content in apple wine ranges between 11 to 14%. Before fermentation apple juice is supplemented with cane sugar. Sometimes ammonium chloride or phosphate are also supplemented in the juice for fermentation.

ii) Custard apple

Annona squamosa is a tropical fruit. Since it is rich in fermentable sugars and delicate flavour, its pulp after the removal of seeds and skin can be used for wine making. The juice obtained is ameliorated to 23^{o} brix and acidity 0.7%, phosphate 0.05% and 125 ppm SO_2 and nitrogenous salts. The must (juice) is inoculated with *Saccharomyces cerevisiae* and fermentation is carried out.

iii) Pear wine

Perry or pear wine of good quality is prepared from pears with high tannin contents. Since it is deficient in nitrogen, an exogenous addition of nitrogen is necessary. Perry fermentation is carried out at temperature of 20-22^{o}C.

iv) Mango wine

Mangoes (*Mangifera indica* Linn.,) is a tropical fruit extensively cultivated in India. Mango fruits and several processed preparations are exported to different countries. It contains high amounts of total solids, vitamins and minerals. Among the numerous varieties 'Safaida, Dashehari, Langra, Chaussa of U.P., Alphanso of Ratangiri, Badami of Mysore, Raspuri and Mulgoa of Tamilnadu and Karnataka are rich in total soluble solids and also in fermentable sugars (glucose, fructose and other sugars). In majority of mangoes, juice extraction is a problem and therefore pectinolytic enzymes are suitably added and the sugar level is raised to 20^{o} brix using cane sugar. Fermentation is done at 22^{o}C with 100 ppm of SO_2. Different varieties of mangoes results in products having varied taste and body.

v) Jamun wine

Jamun fruit is also native to Indian fruit and grown in every part of the country. Jamun fruits have therapeutic properties especially in diabetes and its products have wide acceptability everywhere. This fruit is also deficient in nitrogen, rich in pectin and hence amelioration with sugar (23^{o}Brix) diammonium hydrogen phosphate (0.2%), 0.25% pectinolytic enzyme and 150 ppm of SO_2 is required. The other factors remain the same as with other fruits (grapes etc.,) Popularization of the product is needed for indigenous and export purposes.

vi) Coconut and Palm wine or Toddy

Toddy is an alcoholic beverage produced from the sap of palm trees (*Acrocomia mexicana*) and coconut inflorescence sap. It is consumed in several parts of the country. The sap is collected in clay pots. The middle aged (fully grown) tree is selected and triangular or rectangular cut is made on the top of the tree and the earthen pot is hanged. The foamy sap flows and collects in the pot in the morning and evening. Freshly collected sap is sweet, relishing and invigorating. The fermentation starts as soon as sap starts collecting in the pot. The palm sap contains 10-12% sugar whereas coconut sap generally has about 15-18% sugars. Mostly toddy contains about 6-7% of ethanol. Natural fermentation takes place by the microorganisms present in the earthen pot. *Saccharomyces cerevisiae* is mostly present along with *Lactobacillus plantarum* and *Leuconostoc mesenteroides*. If fermented sap "toddy" is stored for longer time, acetic acid production ensues resulting in the spoilage of the product.

vii) Pomegranate and other fruit wines

Pomegranate (*Pumica grantum)* fruits are pressed as such to get astringency in the wine. Sugar is ameliorated in the pressed juice getting brix around 22-23^{o}B. Potassium metabisulphite is added to the must and it

is inoculated with the starter culture (*Saccharomyces cerevisiae)* at the rate of 5%. Wine is produced in the California belt of the United States of America.

Litchi, Apricot, Pineapple, Date, Red Raspberry, Strawberry, Kiwi fruit, peach, Kinnow fruits, grape fruit, orange and plum, ber, guava and banana fruits can be used for wine production with certain modifications in the method of preparation. Majority of these fruits require enzymatic treatment to release the sugars and supplementation of juice with cane sugar and nitrogeneous salts. The other steps remain the same as followed for production of grape wine.

viii) Mead

Mead is a wine prepared from honey. The procedure followed for mead is the same which is employed for wine making from different types of fruits. Light coloured honey is preferred for this purpose. Honey is diluted to 22-23^{o}Brix, it is boiled and cooled and appropriately tannin, acid and diammonium hydrogen phosphate and SO_2 added to it. The must is inoculated with the active starter culture of *Saccharomyces cerevisiae* and fermentation is carried out at low temperatures. Mead is relished in European countries and several other parts of the world.

12.11 CHEMICAL COMPOSITION OF WINE

The chemical composition of the wine relates to its quality. There are several factors which influence the quality of wine. The most important factors affecting the quality have already been highlighted earlier. The typical wine contains ethyl alcohol, sugars, acids, higher alcohols, tannins, aldehydes, esters, amino acids, minerals, vitamins, anthocyanins, fatty acids, flavouring compounds and traces of methanol.

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. Explain how wine is produced from these fruits: apples, custard apples, pear, mango and jamun.

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2. Define toddy and explain its preparation.

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3. What do you know about mead?

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4. Do you know the typical chemical composition of wine?

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12.12 LET US SUM UP

Alcoholic beverages have been consumed all over the world since time-immemorial. Their (wine, beer, brandy, whisky, rum) consumption is gradually and streadily increasing in developed and developing countries equally inspite of several restrictions. Wine and beer if taken moderately, have health giving properties. When fruit juices (grapes, apple, pear etc.,) are allowed to spontaneous alcoholic fermentation or with addition of yeast culture, the sugar present in these get converted into ethanol and carbon dioxide and thus wine, cider, perry and if distilled, brandy are produced. The yeast, *Saccharomyces cerevisiae* or *Saccharomyces cerevisiae* var. *ellipsoideus* are primarily responsible for good alcoholic fermentation. The fermentation of fruit juices

initially requires some oxygen for the growth of yeast and after its exclusion, ethanol and carbon dioxide are produced anaerobically. Several prefermentative and post fermentative practices are followed and when fermentation is complete, it is clarified with bentonite or gelatin. Maturation of wine (clarified and filtered fermented juice) is carried out in oak wood barrels and finally it is bottled in dark amber colour glass bottles. Apples, grapes, pears, mango, pomegranate, jamun can be utilized for wine production. Honey is used for mead production. Toddy another alcoholic beverage of India, has good invogirating and relishing effect if taken directly from the tree without any storage.

12.13 KEY WORDS

Wine	**:**	Alcoholic fermented juice of fruits
Cider	**:**	Wine from apples
Perry	**:**	Wine from pears
Mead	**:**	Wine from honey
Brandy	**:**	Distilled wine
Rum	**:**	From fermented molasses or canesugar
Whisky	**:**	Distillation of mash of malted grains
Beer	**:**	From malted barley
Toddy	**:**	Palm trees sap or coconut influorescence sap

12.14 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   - The juice of grapes or any other fruits if allowed to ferment by natural fermentation or by addition of starter culture viz., yeast for some time and the resultant product after fermentation is wine.
   - Types of Wines are:
     - Champagne: foaming containing dissolved oxygen.
     - Sparkling wine: produced by injecting carbon dioxide into the wine.
     - Liqueur Wine: Sweet with higher content of alcohol.
     - Sherry: Fortified wine.
     - Port: Wine is stored in a wooden cask.
     - Vermouth: Flavoured wine
     - Brandy: Distilled wine
     - Cider: Produced from apple.
     - Perry: Produced from pears.

2. Your answer should include the following points:

   - The juice of grapes or some fruits contain high amounts of sugar mainly glucose and fructose sometimes 20-25%. The wine yeast present in the juice or added separately grows profusely and convert sugars into ethanol.
   - One mole of glucose gives rise two moles of ethanol and two moles of carbondioxide.
   - Usually 8-10% ethanol is obtained after fermentation.

3. Your answer should include the following points:

   - Grape juice or any other fruit juice.
   - The commercially used yeast strains for wine *Saccharomyces cerevisiae, Saccharomyces cerevisiae* var. *ellipsoideus*.

4. Your answer should include the following points:

   - Killer yeast/s infected with a virus that kills the wine yeast.

5. Your answer should include the following points:

   - *Saccharomyces cerevisiae* strains tolerate high levels of sugars in the fruit juices.
   - Tolerate high levels of ethanol.
   - Grow even at low pH.
   - Ferment fruit juices at low temperature.
   - Resistant to sulphur dioxide which checks the growth of bacteria, yeasts, moulds.

6. Your answer should include the following points:

   - Stemming, crushing and juice separation → clarification → adjustment of sugar level and acidity → fermentation

7. Your answer should include the following points:

   - The growth of wine yeast: *Saccharomyces cerevisiae* is checked due to the presence of Killer viruses and fermentation does not proceed well. A number of metabolites (chemicals) are released causing defective flavour and body of wine (Stuck Fermentation).
   - Clarifications and Stabilization, fining, centrifugation, filteration, removal of crystals, avoidance of haze formation maturation and bottling.

Check Your Progress Exercise 2

1. Your answer should include the following points:

   - Cider (A low alcoholic 4-6% ethanol) from apples.
   - Custard apple seeds and skin are removed and juice is supplemented with sugar and fermented.
   - Perry or pear wine from pears prepared by extra addition of nitrogen in the juice.
   - Juice extraction a big problem and therefore pectinolytic enzyme is used for the recovery of juice and which is supplemented with sugar and fermented (mango).
   - Jamun wine has therapeutic properties and good for diabetes patients.

2. Your answer should include the following points:

   - An alcoholic beverage produced from palm and coconut trees.
   - The sap is collected from the trees in the morning.
   - Freshly collected sap is sweet, relishing and invigorating.

3. Your answer should include the following points:

   - Prepared from honey.
   - Honey appropriately diluted and supplemented with tannic acid and phosphate and nitrogenous salts and fermented.

4. Your answer should include the following points:

   - Contains usually ethyl alcohol, sugar, acids, higher alcohols, tannins, aldehydes, esters, amino acids, minerals, vitamins, anthocyanins, fatty acids, flavouring compounds and traces of methanol.

12.15 SOME USEFUL BOOKS

1. Boulton, R.B., Singleton, V.L., Bisson, L.F., and Kunker, R.E. (1995) Principles and Practices of Wine Making. Chapman and Hall, New York.

2. Jackson, R. (1994) Wine Science: Principles and Applications. Academic Press, San Diego.

3. Zoecklein, B.W., Fuselang, K.C., Gump, B.H., and Nurry, F.S. (1995) Wine Analysis and Production. Chapman Hall, New York.

UNIT 13 TECHNOLOGICAL ASPECTS OF INDUSTRIAL PRODUCTION OF ALCOHOLIC BEVERAGES AND RELATED PRODUCTS

Structure

13.0 OBJECTIVES

After studying this unit, you should be able to:

- state the necessity of technology in alcoholic beverages production;
- describe the vats and tanks used for industrial production of alcoholic beverages;
- know how different types of beverages are manufactured and various steps involved in their production; and
- describe the method of vinegar production.

13.1 INTRODUCTION

In Unit 10, the industrial significance and necessity of fermentation and its relevance to the society have been highlighted in detail. Fermentation or production of wine, beer, cheese or bread is an art and has been practiced from time immemorial throughout the world. However, to meet the demand of the society, large scale operations are needed for any type of product. Therefore, for all the stages of commercial production, engineering involvement is warranted. The scientific knowledge on production of a product either developed in research laboratories or house-holds or cottage level, has to be developed in the form of a commercial process with the help of engineers, scientists and other experts. There are a number of commercial processes developed for alcoholic beverages and related products, and improvement in the quality and also cost reduction of the final products are made more often.

In brief, various aspects of industrial production of selected wines, distilled alcoholic beverages of fruit origin (Brandy) and vinegar are highlighted here.

13.2 FERMENTERS

In wine production, any non-porous, non-toxic vessel can be used as a fermenter. These are constructed of non-aromatic wood, cement, stainless steel or fiberglass. Only two types of fermenters: Vats and Tanks are employed for fermentation. The size of fermenter differs but most of the wineries use 20000 liters and above capacity vessels either open or closed. Sometimes smaller, barrels are employed in which fermentation and maturation both take place. All these are batch fermentation carried out for certain periods and then again recharged with must and restarted. Hydraulic press, filteration equipments and other machines and equipment are used for commercial production.

13.3 TECHNOLOGY FOR CIDER-MAKING

Cider is a low alcoholic drink prepared from the apple juice. Depending on the ethanol content, it is considered soft cider (1-5%) or hard cider (6-7%). In India, cider is not produced commercially. But the Indian varieties of apple cultivated in Jammu and Kashmir and Himanchal Pradesh e.g., Ambiri-Kashmiri, Red Delicious, Gold Pippin, Maharaji Apples and Golden Delicious can be utilized for cider making. A method for cider manufacture at a commercial scale is shown through flow diagram in Figure 13.1.

13.4 TECHNOLOGY OF SPARKLING CIDER

Sparkling cider is prepared by artificial carbonation. The secondary fermentation is carried out for 3-4 days at 21.0 ^{0}C. A protocol for carbonated cider is given in Figure 13.2.

13.5 TECHNOLOGY OF FORTIFIED WINES: VERMOUTH

Wines which are flavoured with herbs and spices and contain ethanol content in the range of 15-21%, are classed under ‘Vermouth’. Both dry and sweet vermouth are prepared in European countries and relished in cocktails. It is prepared by making the wine and then extracting the spices and herbs mixture in wine and Brandy and blending with the wine produced and also adjusting the alcohol level. Grapes, apples apricots, mango, plum, even tamarind can also be used for vermouth production. Pear or similar types of fruit which are produced in abundance and commercially do not find much market, can be profitably used for vermouth manufacture. It is schematically illustrated in Figure 13.3.

13.6 TECHNOLOGY FOR BRANDY-MAKING (DISTILLED ALCOHOLIC BEVERAGES)

Brandy is defined as a distilled alcoholic beverage prepared from the distillation of wine or any fermented fruit juice. Usually brandy denotes the distilled wine of grapes whereas for other fruits it is prefixed with the fruit name e.g. apple brandy, plum brandy etc., Sometimes ‘Cognac’ word is also

used for brandy. Cognac is produced in France. There are many varieties of wines mostly based on their place of manufacture.

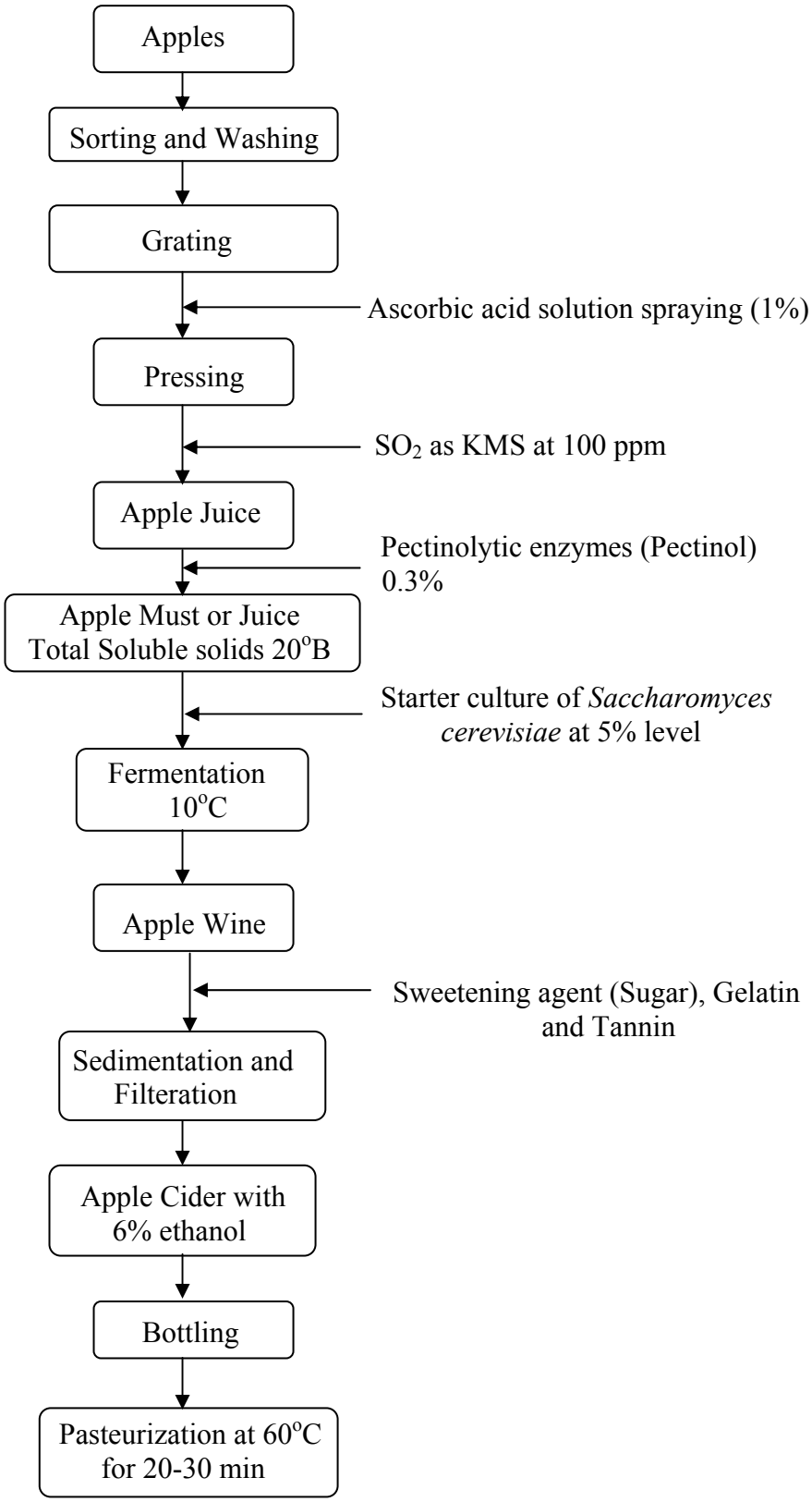

Figure 13.1: A protocol for cider production

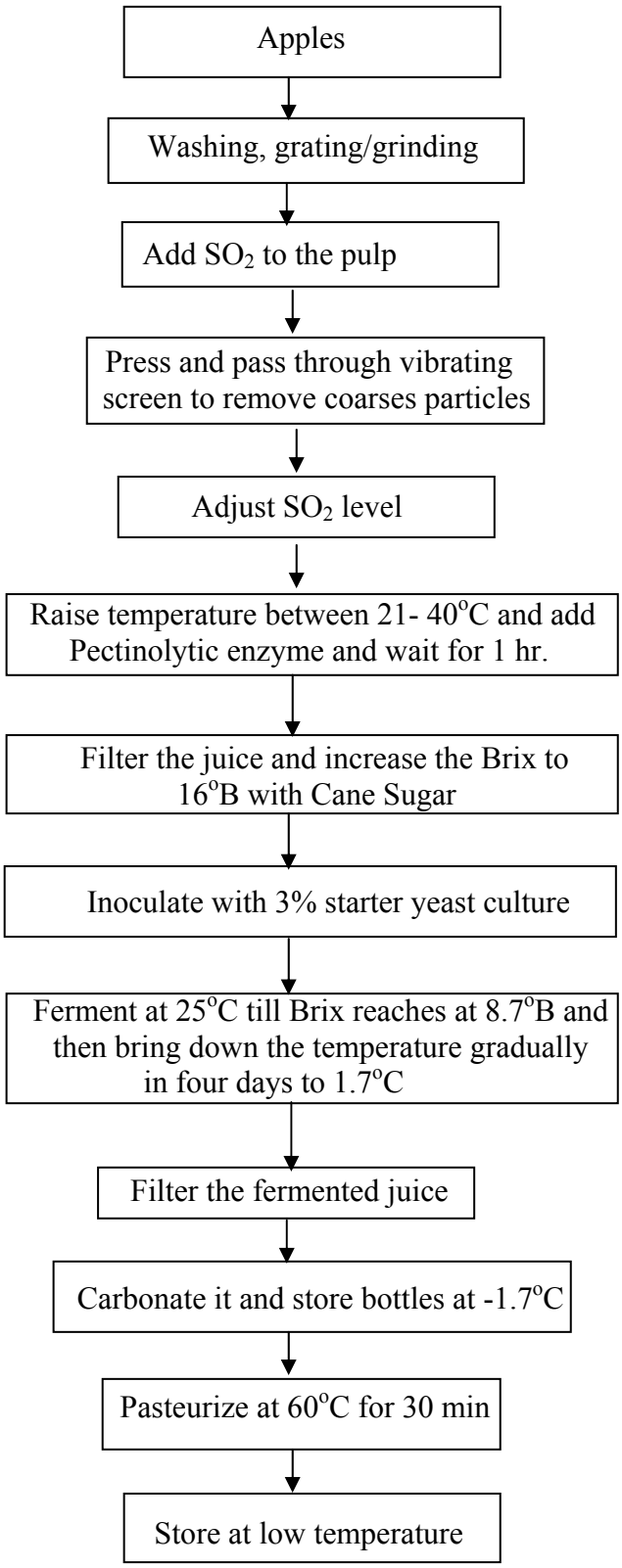

Figure 13.2: A protocol for carbonated cider production

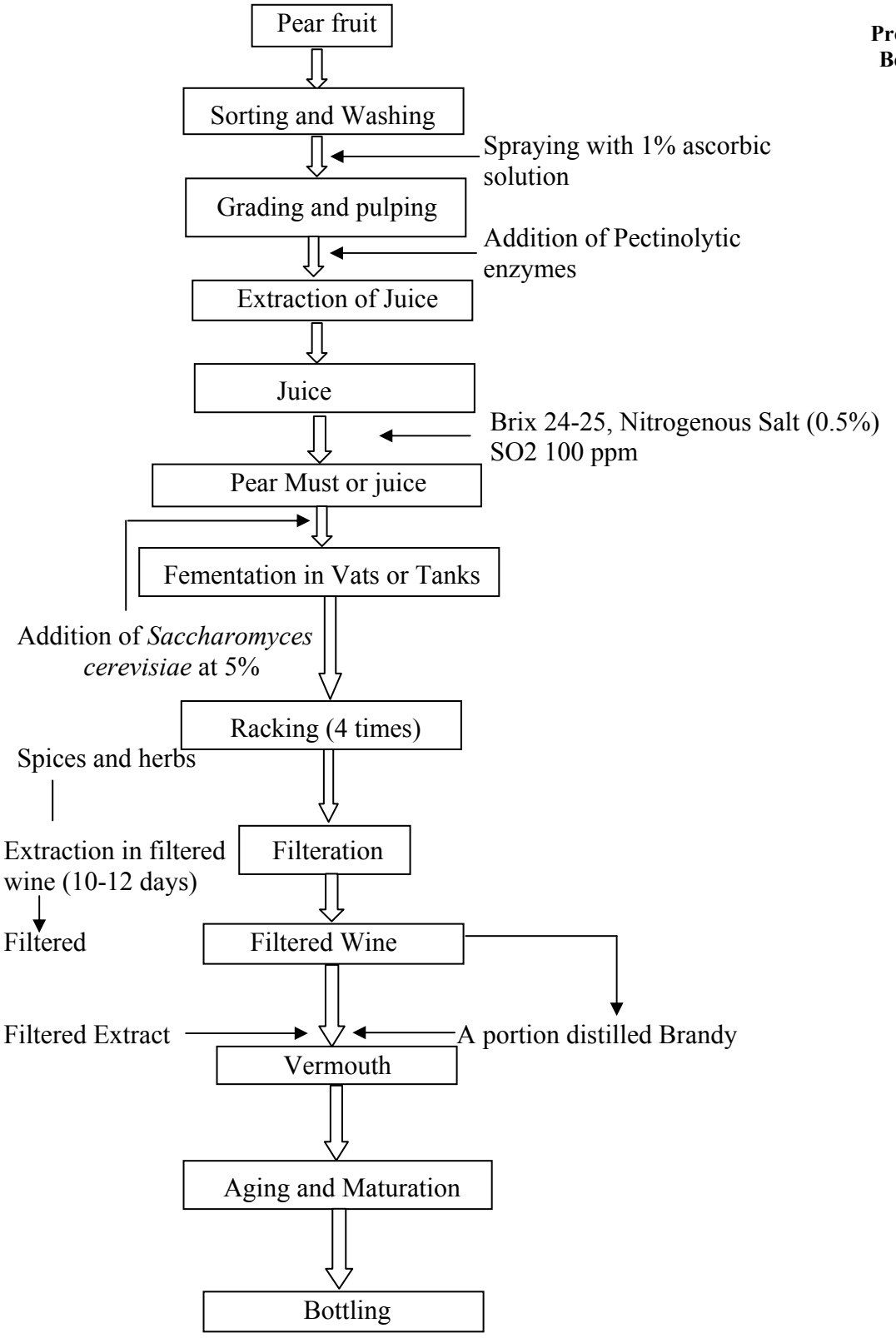

Figure 13.3: Manufacture of vermouth of pears

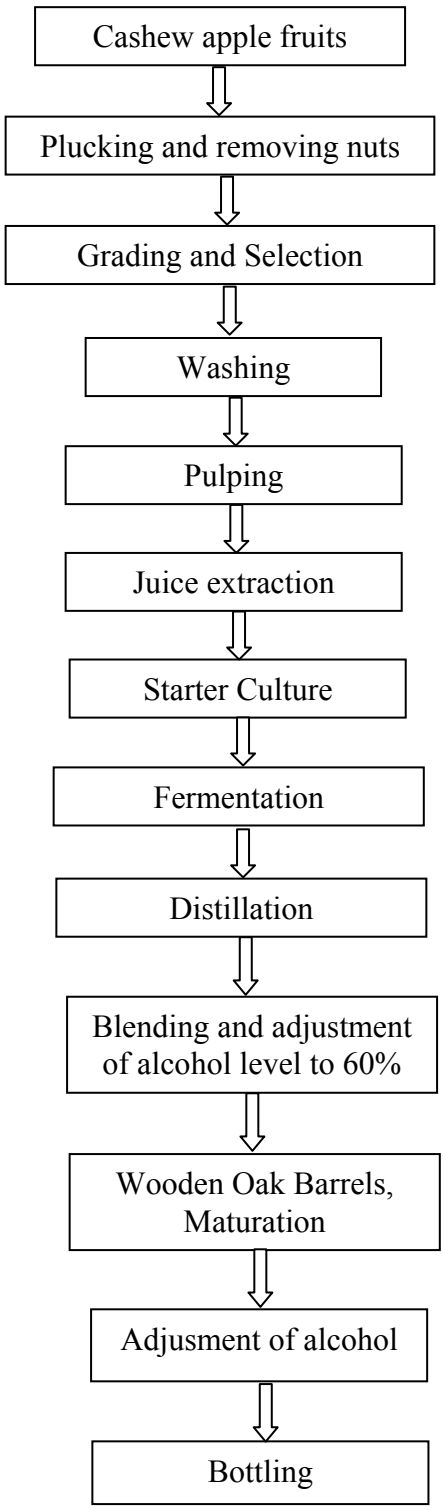

Figure 13.4: Manufacture of Feni from cashew apple

Like wines, brandy also contains many constituents. The major being ethyl alcohol (ethanol) along with fusel oil (higher alcohols e.g. n-propyl alcohol, isobutyl alcohol etc.,), traces of aldehydes esters (ethyl acetate) furfural and several other constituents. The method of base wine production for brandy is as employed for other wine manufacturing.

Distillation of fermented wine is carried out mostly in copper pot distillation unit but column stills (a vacuum distillation) is also adopted in some of the

wineries. Distillation is carried out along with some fermented fruit materials and yeast cell biomass (lees). It is done in two steps. The first step results in about 28% of ethyl alcohol in the distillate whereas in the second stage of distillation of the distillate yields 70% of ethanol by volume. Brandy is stored in oak barrels. Some of the products are stored for several years e.g. 15 to 50 years and the quality improves during maturation. The final product has mostly 40-45% of ethyl alcohol by volume.

In the United States of America and several other countries, the strength of alcohol is measured in terms of proof. It denotes usually the twice the percent of ethyl alcohol content of a liquid at 60^{o}F.

13.7 TECHNOLOGY OF FENNY AND BRANDY OF CASHEW APPLE

Cashew apple is grown in large areas of the western coast of Karnataka, Goa and other states for cashew nuts. The nut is attached to the fleshy fruit and while processing, the fruit pulp is mostly wasted or a part is utilized for wine-making. It is rich in fermentable sugars and ascorbic acid. In Goa 'Kaju Feni' is quite popular. In Tanzania, Brazil and several other countries, alcoholic beverages are produced and used as a wine or occasionally distilled and consumed in the form of brandy. The word 'Feni' comes from the Konkani language, which means 'froth'. It is mostly produced at a cottage scale level. However, technical know-hows are available for commercial production of feni and brandy from it.

The cashew apple fruits are crushed and pulped and juice is extracted using extractors. The juice is left as such for spontaneous natural fermentation or inoculum of *Saccharmyces cerevisiae* at 5% is added, stirred and mixed evenly. Normally 2-3 days are taken for the fermentation. The feni is obtained by distilling the fermented juice with Urak* (2:1) containing 60% alcohol. Aging and maturation at 15^{o}C is carried out in oak wood barrels. After aging the product is marketed in bottles adjusting alcohol content to 42-43%.

The process of feni production is illustrated in Figure 13.4.

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 is technology necessary for the production of alcoholic beverages?

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* Urak: Distilled feni containing 60% alcohol

2. What special type of fermenters needed for wine production?

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3. What are the important characteristics of sparkling cider and vermouth?

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4. Highlight the importance of brandy among alcoholic beverages.

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5. What do you mean by 'Fenny'? Write its method of production.

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13.8 TECHNOLOGY OF VINEGAR PRODUCTION BY FERMENTATION

Vinegar is consumed in almost every part of the world. Sugars mostly glucose, fructose and sucrose in appropriately diluted concentration are converted to ethyl alcohol by yeast/s and acetic acid producing bacteria convert this alcohol into ethanol by acetification thus producing vinegar. Vinegar contains at least 4% of acetic acid with some other solids. It is made from distilled alcohol prepared from cane molasses or other sugar sources. According to Indian Standards Vinegar should not contain arsenic, mineral acid, lead, copper, or colouring matter except caramel.

i) Method of Vinegar Preparation

Two distinct processes are involved in the preparation of vinegar:

a) Transformation of sugars of fruits or any sugary materials into alcohol by yeast anaerobically;

b) conversion by acetic acid producing bacteria of alcohol oxidatively in the presence of air into acetic acid.

$$\underset{\text{Fermentable Sugar}}{C_6H_{12}O_6} \xrightarrow{\text{Yeast}} \underset{\text{Ethylalcohol}}{2C_2H_5OH} + \underset{\text{Carbon dixoide}}{2CO_2}$$

$$\underset{\text{Ethyl alcohol+ Oxygen Sugar}}{2C_2H_5OH + 2O_2} \xrightarrow[\text{Producing bacteria}]{\text{Acetic acid}} \underset{\text{Acetic acid}}{2CH_3COOH} + \underset{\text{water}}{H_2O}$$

ii) Types of Vinegar

Cider Vinegar (apple juice)
Wine Vinegr (grape juice)
Spirit Vinegar (Dilute ethylalcohol from molasses)
Malt Vinegar (barley malt)
Orange, pineapple, banana, pear, peach, apricot, onion vinegars

iii) Process of Vinegar Preparation

There are a number of processes commercially used for vinegar production. The most commonly used are:

a) Slow process: The juice or sugary solution is filled into barrels and allowed to undergo alcoholic fermentation and acitification slowly. The mouth of the barrel or container is covered with a piece of cloth and placed in a damp and warm place. In about 5 to 6 months, the sugar solution turn into vinegar. This process is time-taking.

b) Oreleans Slow Process: The barrel used is filled three-fourth with the juice and two holes above the juice level on the either side of barrel are made in addition to top hole or mouth. These holes are tightly covered with cheese cloth to protect from insects and flies. The filled barrel is placed in a warm place (21-27^{o}C) and fermentation is allowed to take place. It takes about 3 months for complete fermentation. About three fourth of the fermented liquid (Vinegar) is withdrawn without making any hole in the top thick film of *Acetobacter* and again filled with the fresh juice.

c) Quick Process: It is known as the 'Generator' or the 'German' process. The generator is in the form of a cylinder 3.6 to 4.2 meters high and 1.2 to 1.5 meters in diameter. It has a false bottom and head, vent holes and sparge for discharging the liquor. It has three compartments. The

central compartment is filled with beech wood shavings, corn cobs, pumice stone, straw to increase the surface area for the growth of acetic acid producing bacteria. The other distribution compartment is above 30 centimeters of the central compartment. It is separated by a partition perforated plate having a number of holes. A revolving sprinkler or a fitting trough is fitted in order to allow liquid to trickle slowly over the shavings of the Central Compartment. The third compartment is the bottom compartment separated from the central compartment by a perforated partition above 1.5 Meters the bottom of the generator.

Initially beech wood shavings are wetted with unpastuerized vinegar and then two parts of alcoholic juice and one part of vinegar is charged slowly to allow bacteria to colonize the shavings. When the generator becomes ready then alcoholic juice or liquid is passed through the generator on the top and acetified liquid (Vinegar) is collected having 3 to 4% acetic acid (volume). The air passage is cleaned from time to time. The optimum temperature is around 27-30^{o}C for this operation.

d) *Fringe Process:* The quick process has been improved by the addition of forced aeration and temperature control of trickling generators.

e) *Submerged Culture Process:* It has been tried for Vinegar production but *Acetobacter aceti* is quite sensitive to oxygen. Therefore, the aeration of the liquid is significantly important. However, it is 80 times faster oxidation of ethanol to acetic acid.

iii) Post-fermentation Process

a) *Aging:* Vinegar produced by quick process is kept for aging for about six months.

b) *Clarification:* Before bottling, vinegar is made sparkling clear.

c) *Pasteurization:* It is heated in an open vessel to about 60^{o}C and then cooled and bottled.

d) *Spoilage of Vinegar:* Lactic acid producing bacteria grow initially and spoil the vinegar. It should be fully avoided. Film yeast growing on the top also spoil the process of vinegar generation. Vinegar flies are well known to affect the quality of vinegar. Vinegar eels, louse and vinegar mites are the enemies of vinegar production. They should be completely eliminated.

At present a large proportion of our demand for vinegar is being met by synthetic vinegar. However, the fruit vinegar may be encouraged for consumption. People are not familiar with fruit vinegars and therefore, these have to be popularized highlighting their therapeutic importance in human health.

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. Explain the importance of vinegar.

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2. What are the steps involved in vinegar generation and types of vinegar produced?

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3. List commercial processes used for Vinegar generation.

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13.9 LET US SUM UP

Production of exotic beverges in smaller quantities is an art but to meet the increasing demand of the people, large-scale operations at commercial scales are required. Therefore economically viable technologies are warranted for industrial production. All the beverages are commercially produced and marketed all over the world. Commercial vats or tanks with juice extractor and filteration or clarifier units are employed for these alcoholic beverages. Brandy manufacture requires a distillation unit along with the modern bottling plant. Vinegar is also a commercial fermentation produced throughout the world. There are a number of quick processes which take considerably shortertime for vinegar generation.

13.10 KEY WORDS

Vats and Tanks : Open or closed mouth vessels for wine fermentation.

Sparkling cider : Artificially carbonated cider.

Vermouth : Wine flavoured with herb and spices containing 15-21%, ethanol.

Brandy : Distilled wine or fermented fruit juice.

Fenny : Wine from cashew apple pulp.

Vinegar : Prepared from sugary materials by fermentation containing 4 grams of acetic acid/100 ml.

13.11 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   - It is an art at house-hold or small scale production.
   - For Commercial production, large scale operation needed.
   - For large scale operations, engineering inputs needed for fermentation, filtration, maturation and bottling.

2. Your answer should include the following points:

   - Unlike other fermentations (antibiotic, citric acid), non porous and non-toxic vessels are used.
   - They are constructed from non-aromatic wood, cement, stainless steel or fibre glass.
   - Vats and tanks used for wine fermentation.

3. Your answer should include the following points:

   - Prepared by carbonation.
   - Secondary fermentation done at lower temperatures.
   - Wine which flavoured with herbs and spices and contain ethanol in the range of 15-21%: vermouth.

4. Your answer should include the following points:

   - Distilled wine or any fermented juice (brandy).
   - Mostly 40-45% ethanol volume (brandy).

5. Your answer should include the following points:

   - Produced from fleshy pulp
   - Sorting of pulp → extraction of juice → allowing spontaneous fermentation or addition of starter culture for 2-3 days → distillation of the fermented juice.
   - Distilled product contains 42% ethanol.

Check Your Progress Exercise 2

1. Your answer should include the following points:

   - A fermentation derived product, containing not less than 4 grams of acetic acid per 100 ml.
   - Prepared in the household in small quantities from sugarcane juice in India or cider or wine of fruits.

2. Your answer should include the following points:

   - Two distinct processes: the first being sugar fermentation to ethyl alcohol by *Saccharomyces cerevisiae* and the next being conversion of ethyl alcohol to acetic acid by *Acetobacter* spp.
   - Commercially available vinegar: Vinegar from jaggery, vinegar from cider, Barley malt vinegar.

3. Your answer should include the following points:

   - Slow process
   - Oreleans process
   - Quick process
   - Fringe process
   - Submerged process

13.12 SOME USEFUL BOOKS

1. Boulton, R.B., Singleton, V.L., Bisson, L.F., and Kunker, R.E. (1995) Principles and Practices of Wine Making. Chapman and Hall, New York.

2. Jackson, R. (1994) Wine Science: Principles and Applications. Academic Press, San Diego.

3. Zoecklein, B.W., Fuselang, K.C., Gump, B.H., and Nurry, F.S. (1995) Wine Analysis and Production. Chapman Hall, New York.

EXPERIMENT 1 DETERMINATION OF ACIDITY AND pH

Structure

1.1 INTRODUCTION

Acidity of foods is usually determined by acid, base titration using standard sodium hydroxide. The reaction being between a weak acid and a strong alkali, phenolphthalein is used as the end point colour indicator, which produces a faint pink colour around pH 8. For dark coloured solutions, alkali titration can be carried out to pH 8.1 using a pH meter.

pH of foods is determined using a pH meter having glass electrode and calomel electrode or a combination electrode.

Objectives

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

- determine the acidity of food products by alkali titration; and
- determine the pH of the product using a pH meter.

1.2 EXPERIMENT 1a: DETERMINATION OF ACIDITY

1.2.1 Principle

Organic acids react with sodium hydroxide to form their corresponding sodium salts. The common organic acids are mono- carboxylic (acetic acid, lactic acid), dicarboxylic (malic and tartaric acids) and tri- carboxylic (citric acid) acids. The general reaction between an organic acid and sodium hydroxide is as follows.

$$R–(COOH)n + n\ NaOH = R–(COONa)n + n\ H_2O$$

By knowing the equivalent weight of the acid, the acid content can be calculated based on the alkali required for neutralization.

1.2.2 Requirements

Equipment and Apparatus

Chemical balance

Conical flask – 250 ml –3

Beaker – 100 ml –1

Volumetric flask – 100 ml –1

Burette – 10 ml –1

Pipette – 5 and 10 ml –1 each

Chemical and Reagents

Sodium hydroxide solution – 0.1 N

Phenolphthalein indicator – 0.1% in alcohol

1.2.3 Procedure

Acidity determination
Sample preparation

Thin Juices, RTS beverages etc.: Mix thoroughly and filter through previously washed and dried muslin cloth. Use 5-10 ml for titration.

Fresh fruits & vegetables, dried fruits, preserves, jams, marmalades pickles etc.: Pulp the material in a blender or mortar and mix thoroughly. Accurately weigh 10 to 20 g of the pulped material in a beaker, add about 50 ml distilled water and boil gently for 15 to 30 min replacing the water lost by evaporation. Cool, transfer to a volumetric flask (say 100 ml) and make up the volume. Filter through Whatman No.1 filter circle, if necessary.

Fruit pulps, squashes, syrups, cordial etc.: Weigh 10-20 g of the material, mix with distilled water, heat on steam bath to dissolve, cool and make up the volume in a volumetric flask (say 100 ml).

Pipette out suitable aliquot (5-10ml) of the prepared sample (quantity depending on the acidity of the sample) into a 250 ml. conical flask. Add about 50 ml of distilled water and few drops of phenolphthalein indicator. Titrate to light pink end point with 0.1 N NaOH solution. Products like juices and beverages may be directly weighed (5-10g) and transferred into 250 ml conical flask with about 50 ml distilled water and titrated.

1.2.4 Observations

Where samples are boiled with water and made up to volume:

Weight of sample	= W	= ---------- g
Volume made up to	= V_1	= ----------ml
Volume of aliquot taken for titration	= V_2	= ----------ml
Volume of NaOH required	= V_3	= ---------- ml
Normality of the NaOH solution	= 0.1	

Where sample is weighed and directly taken for titration:

Weight of sample = W_1 = ---------- g

Volume of NaOH required = V_4 = --------- ml

Normality of the NaOH solution = 0.1

1.2.5 Calculations

1000 ml 1N NaOH = One gram equivalent of organic acid.

Calculate the acidity in terms of the predominant acid present in the product. The equivalent weights of some common organic acids and the foods in which they are the major acids are given below. However, unless specifically required, it is customary to calculate the acidity of food materials as anhydrous citric acid.

Organic acid	**Equivalent Wt. (E.W.)**	**Foods**
Anhydrous citric acid	64	Citrus fruits
Malic acid	67	Raw mango, apple
Tartaric acid	75	Grapes, tamarind
Lactic acid	90	Milk foods
Acetic acid	60	Vinegar containing foods

For samples boiled with water and made up to volume:

$$\% \text{ acid} = \frac{\text{E.W. of acid} \times \text{Titer} \times \text{Normality of NaOH} \times \text{Volume made up} \times 100}{1000 \times \text{Aliquot taken} \times \text{Weight of sample}}$$

$$= \frac{\text{E.W. of acid} \times V_3 \times 0.1 \times V_1}{10 \times V_2 \times W}$$

For sample taken directly for acidity estimation:

$$\% \text{ acid} = \frac{\text{E.W. of acid} \times V_4 \times 0.1}{10 \times W_1}$$

For carbonate beverages, expel carbon dioxide by warming or just heating to boil, cool and then titrate.

1.2.6 Result

Acidity of the given product = Percent (w/w)

1.3 EXPERIMENT 1b: DETERMINATION OF pH

1.3.1 Principle

Acidity of any solution is dependant on the pH and in turn pH depends on the Hydrogen ion concentration of solution. pH can be calculated by the equation pH = negative logarthim of Hydrogen ion concentration i.e.,

$$pH = -\log (H^+)$$

1.3.2 Requirements

pH meter

Buffer solutions – pH 4.0, 7.0 and 9.0

1.3.3 Procedure

Sample preparation

Liquid samples are used as such for pH measurement. Solid and semi-solid materials have to be homogenized well before the measurement.

Procedure

The pH meter should be switched on for at least 30 min before taking measurements for stabilization. The pH meter is calibrated against standard buffers before measuring the pH of sample. pH meters are provided with operating instructions, which should be followed. Rinse the electrode(s) with water and wipe dry using a filter paper in between buffer and sample pH measurements.

1.3.4 Results

The pH of the sample is recorded usually to the first decimal place

1.4 PRECAUTIONS

The general precautions mentioned in the course ‘Introduction’ and those indicated in the experiments should be followed meticulously.

EXPERIMENT 2 DETERMINATION OF MOISTURE

Structure

2.1 INTRODUCTION

Moisture content of vegetables and most of their products can be determined by drying the material in an air oven. A fairly high temperature of the order of 105^0C is required to remove the bound water in foods. However, at that temperature, foods containing appreciable proportion of sugars like fruit products decompose giving wrong results. Therefore, it is advisable to dry such products at lower temperature, such as 60-70^0C, preferably in a vacuum oven.

Objectives

After performing this experiment, you should be able to:

- determine the moisture content of foods by air oven method; and
- determine the moisture content of sugary products by vacuum oven method.

2.2 EXPERIMENT

2.2.1 Principle

Moisture in foods exists both as free water and bound water. Bound water is more difficult to remove by heat. Therefore, the time required for complete removal of water from a food material varies. Hence drying has to be continued till constant weight is reached.

2.2.2 Requirements

Apparatus

Hot air oven (thermostatically controlled)	–1
Vacuum oven	–1
Chemical balance, 1 mg sensitivity	–1
Desiccator (with active desiccant)	–1

Moisture dishes with tight fitting lids –6

Aluminium dishes (7 cm. Dia.)
Sea sand (washed and ignited)
Glass rods

2.2.3 Procedure

a) Air oven method

Weigh accurately 5 g of the material in a dish previously dried and weighed. Place the dish along with lid in an electric air oven maintained at 70^0C in the case of fruits or their products, or 100^0C in the case of vegetables or their products. Cool the dish to room temperature in a desiccator and weigh with the lid on. Repeat the process of heating, cooling and weighing until the loss in weight between two successive weighings do not vary by more than 3-5 mg. For most of the samples 16-18 hr heating is sufficient. Record the lowest weight obtained.

b) Vacuum oven method

Place 20-25 g pure sea sand and a short glass rod in an aluminium dish having a tight fitting cover. Dry thoroughly and cool in a desiccator and weigh. Accurately weigh 5 g sample and transfer completely to the dish by rinsing with water. Mix well with the glass rod and heat on steam bath for partial drying. Transfer the dish to vacuum oven and dry the sample at 70^0C at a pressure not more than 100 mm Hg pressure. Cool in a desiccator and weigh. Repeat the process of drying, cooling and weighing until consecutive weighings made at intervals do not vary by more than 3 mg. Drying for 6 to 7 hr is generally found sufficient.

2.2.4 Observations

a) Air oven method

Weight of the weighing dish with lid = W_1 = ------- g

Weight of the dish with lid and material = W_2 = ------- g

Weight of the dish with lid and dried material = W_3 = ------- g

b) Vacuum oven method

Weight of the dish with sand and glass rod = W_1 = ------- g

Weight of the dish with glass rod and material = W_2 = ------- g

Weight of the dish with glass rod and dried material = W_3 = ------- g

2.2.5 Calculations

a) Air oven method

Weight of the material = (Weight of the dish with sample – weight of the dish)

= $(W_2 – W_1)$ = ------- g

Quantity of moisture in the material = (Weight of the material before drying – weight of the material after drying)

$= (W_2 - W_3) =$ ------- g

$$\text{Per cent moisture in the material} = \frac{\text{Quantity of moisture in the material}}{\text{Weight of the material}} \times 100$$

$$= \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100 = \text{g/100g or \%}$$

b) Vacuum oven method

Weight of the material $= W_2 - W_1$ = ------- g

Quantity of moisture in the material $= W_2 - W_3$ = ------- g

$$\text{Per cent moisture in the material} = \frac{\text{Quantity of moisture in the material}}{\text{Weight of the material}} \times 100$$

$$= \frac{(W_2 - W_3)}{(W_2 - W_1)} \times 100 = \text{g/100g}$$

2.2.6 Results

Moisture content of the sample = ---------- Percent (%) by weight

2.3 PRECAUTIONS

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.

EXPERIMENT 3 DETERMINATION OF ASH AND ITS CHARACTERISTICS

Structure

3.1 INTRODUCTION

Ash content of foodstuffs represents inorganic residue remaining after destruction of organic matter. Acid- insoluble ash is a measure of sand and other silicious matter present. High ash content and/or low alkalinity of the ash in some cases could be due to the presence of adulterants.

Objectives

After studying and performing the experiments, you should be able to:

- determine the total ash content in
  - water-soluble ash;
  - acid-insoluble ash; and
  - the alkalinity of the ash in food products.

3.2 EXPERIMENT 3a: DETERMINATION OF TOTAL ASH

3.2.1 Principle

When a sample of a food material is ashed around 525^0C, the organic matter present is decomposed and expelled. The residue remaining is the mineral matter. The ash content is expressed as per cent weight by weight.

3.2.2 Requirements

Equipment and Apparatus

Silica dish (100ml cap.)
Chemical balance, 1mg sensitivity
Hot plate or burner
Muffle furnace
Desiccator (with an active desiccant)

Precautions

Do not transfer the hot silica dish directly from the muffle furnace to the desiccator. Cool to 100^oC and transfer to desiccator.

3.2.3 Procedure

Note the tare weight of three silica dishes. Accurately weigh 5 g of sample into each. Char the material carefully on a burner or hot plate and transfer the dishes to a muffle furnace and ash at a temperature of around 525^0C until a white ash is obtained. Moisten the ash in dishes with water. Dry on steam bath and on hot plate and re-ash at 525^0C. Cool in a desiccator and weigh. Reserve the ash in one dish for determination of water-soluble and water-insoluble ash, in second dish for acid-insoluble ash, and the ash in the third dish for determining alkalinity of ash.

3.2.4 Observations

Weight of silica dish = W_1 = ------- g

Weight of the silica dish with sample = W_2 = --- --- g

Weight of the silica dish with ash = W_3 =------- g

Calculation

$$\text{Total ash (\%) in the sample} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100 = \frac{(W_3 - W_1)}{(W_2 - W_1)}$$

If the ash content has to be calculated on dry weight basis:

$$\textbf{Total ash content (\%) on dry weight basis} = \frac{(W_3 - W_1) \times 100}{(W_2 - W_1) \times (100 - M)}$$

Where, M is the moisture content (%) of the sample in percent by weight (determined as in experiment 2)

Expression of Results

Ash Content = % by weight or % by dry weight

3.3 EXPERIMENT 3b: DETERMINATION OF WATER-SOLUBLE AND WATER-INSOLUBLE ASH

3.3.1 Principle

When the total ash obtained from the above experiment is boiled in water, part of the ash dissolves (water-soluble ash) and part remains as insoluble ash. The insoluble ash can be estimated gravimetrically. The difference between total ash and water-insoluble ash gives water-soluble ash.

3.3.2 Requirements

Same as used for total ash

Reagents/chemicals

Whatman No. 41 filter circles
Red litmus paper

3.3.3 Procedure

Transfer the ash from one of the three silica dishes, with the aid of about 20 ml distilled water, into a beaker. Cover with a watch glass and boil for 5 min. Filter through an ashless filter paper (Whatman No. 41). Wash the entire residue with hot water until the filtrate no longer turns red litmus blue. (Reserve the entire filtrate for the determination of alkalinity). Dry the ashless paper with residue (water isolubles) in the same silica dish and transfer to muffle furnace and ignite at 525^{o}C for 2 hours. Cool in a desiccator and weigh.

3.3.4 Observations

Weight of silica dish No.1 = W_1 --- g

Weight of the silica dish with sample = W_2 --- g

Weight of the silica dish with total ash = W_3 --- g

Weight of the silica dish with water-insoluble ash = W_4 --- g

3.3.5 Calculations

$$\text{Water-soluble ash (\%)} = \frac{\text{Total ash} - \text{water-insoluble ash}}{\text{Weight of sample}} \times 100$$

$$= \frac{(W_3 - W_1) - (W_4 - W_1)}{(W_2 - W_1)} \times 100 = \frac{(W_3 - W_4)}{(W_2 - W_1)} \times 100$$

$$\text{Water-soluble ash (\%) on dry wt.} = \frac{(W_3 - W_4) \times 100 \times 100}{(W_2 - W_1) \times (100\text{-}M)}$$

Where, M = Moisture % of sample.

3.3.6 Results

Water-soluble ash = % by weight on dry basis.

3.4 EXPERIMENT 3c: DETERMINATION OF ACID-INSOLUBLE ASH

3.4.1 Principle

Acid insoluble ash refers to the portion of ash, which does not dissolve in 1:2.5 HCl under the experimental conditions. This can be estimated either from the total ash (as obtained in Experiment 3a) or water-insoluble ash (as obtained in Experiment 3b).

Preparation of Sample

Use the ash of second disk obtained from the Experiment 3a.

3.4.2 Requirements

Apparatus

Same as used for total ash

Chemicals/ regents

Hydrochloric acid - dilute with distilled water (1: 2.5)
Whatman No. 41 filter circles
Blue litmus paper

Precautions

Do not inhale the vapours of Conc. Hydrochloric acid.

3.4.3 Procedure

To the ash of the second silica dish, add 25ml of hydrochloric acid, cover with a watch glass and boil gently for 5 min. Filter through ashless filter paper. Wash the entire residue with hot water (> 85^oC) until the filtrate no longer turns blue litmus paper red. Dry the ashless paper with the residue in the same dish and transfer to Muffle furnace and ignite at 525^0C for 2 hrs. Cool in a desiccator and weigh.

Alternatively, water-insoluble ash as obtained in Experiement 3b can also be used.

3.4.4 Observations

Weight of silica dish No. 2 = W_1 = --- g

Weight of silica dish with sample = W_2 = --- g

Weight of the silica dish with ash insoluble in acid = W_3 = --- g

3.4.5 Calculations

$$\text{Ash insoluble in acid (\%)} = \frac{\text{Acid-insoluble ash}}{\text{Weight of sample}} \times 100$$

$$= \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100$$

$$\text{Ash insoluble in dilute HCl (\%) on dry wt.} = \frac{(W_3 - W_1) \times 100 \times 100}{(W_2 - W_1) \times (100 - M)}$$

Where, M = Moisture of the sample.

3.4.6 Results

Ash insoluble in dilute HCl = % by weight on dry basis.

3.5 EXPERIMENT 3d: DETERMINATION OF ALKALINITY OF ASH

3.5.1 Principle

Dissolving the total ash in a known volume of standard dilute HCl and titrating the excess acid with standard NaOH determine alkalinity of ash.

Preparation of Sample

Use the ash of the third dish obtained from Experiment 3a.

3.5.2 Requirements

Apparatus

Burette, 10 ml cap.
Conical flask, 250 ml

Chemicals and Reagents

Methyl orange, 0.1% in water
Hydrochloric acid – 0.1N
Sodium hydroxide – 0.1N

3.5.3 Procedure

To the ash in the third silica dish from Experiment 3a, add 10 ml of 0.1 N HCl. Dissolve by warming on a water bath, cool and titrate the excess acid with 0.1 N NaOH using methyl orange indicator. Alkalinity of ash is calculated as potassium carbonate (K_2CO_3).

3.5.4 Observations

Weight of empty dish No. 3 = W_1 = ----- g

Weight of dish with sample = W_2 = ----- g

Weight of dish with ash = W_3 = ----- g

Volume of 0.1 N HCl added to the ash = A = ----- ml

Titre value (ml 0.1 N NaOH) = B = -----ml

Normality of the acid = N

3.5.5 Calculations

One ml of 0.1 N HCl is equal to 0.00691 g of potassium carbonate

Therefore, g of potassium carbonate per g of ash =

$$\frac{\text{ml 0.1 HCl required to neutralize the alkalinity of the ash} \times 0.00691}{\text{Weight of the ash}}$$

$$= \frac{(A - B) \times 0.0061}{(W_3 - W_1)}$$

Alkalinity may also be expressed as number of ml of 0.1 N acid required to neutralize the ash from 100 g of the sample.

Alkalinity of ash from 100 g sample =

$$\frac{\text{ml 0.1 N HCl required}}{\text{Weight of sample}} \times 100 = \frac{(A - B) \times 100}{(W_2 - W_1)}$$

3.5.6 Results

Alkalinity of ash = g of potassium carbonate per g of ash, or ml 0.1 acid required to neutralize ash from 100g sample

3.6 PRECAUTIONS

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.

Use a tongs with long handle for keeping and removing dishes from the muffle furnace.

EXPERIMENT 4 DETERMINATION OF REDUCING SUGARS, TOTAL REDUCING SUGARS, SUCROSE AND STARCH

Structure

4.1 INTRODUCTION

Several methods are available for estimation of reducing sugars. They include chemical, polarimetric and chromatographic methods. However, for routine analysis of food products, Lane and Eynon chemical method is most widely used. Non-reducing sugars and starch are first converted into reducing sugars for estimation.

Objectives

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

- prepare food products for estimation of reducing sugars, total sugars and starch; and
- determine reducing sugars, total sugars and starch in food products by Lane and Eynon method.

4.2 EXPERIMENT 4a: REDUCING SUGARS

4.2.1 Principle

Lane and Eynon method is based on the principle of reduction of Fehling's solution by reducing sugars. Fehling's solution is a mixture of copper sulphate and alkaline Rochelle salt (sodium potassium tartarate). Rochelle salt complexes with the cupric hydroxide formed in alkaline solution and prevent it from precipitation. Reducing sugars reduces the complexed cupric hydroxide to red, insoluble cuprous oxide under the experimental conditions. An oxidation-reduction indicator, usually methylene blue, detects the end point of the reaction.

The first step in the estimation of reducing sugars by Lane and Eynon method is the determination of Factor for Fehling's solution. Fehling factor is the quantity of invert sugar in grams required to completely reduce the Fehling's solution (usually 5 ml each of Fehling's A and B solutions).

Total sugars include reducing sugars and non-reducing di - and oligo-saccharides like sucrose, which on mild acid hydrolysis are converted into reducing sugars. Starch is hydrolysed by strong acids into glucose.

4.2.2 Requirements

Equipment and Apparatus

Chemical balance, 1mg sensitivity

Hot plate

Burette (50 ml cap.) with an off-set tip

Volumetric flask, 250 ml

Pipette, 5 ml and 25 ml

Conical flask, 250 ml

Weighing bottle

Funnel (small)

Whatman No. 1 filter circles

Chemicals and Reagents

Fehling's solution A: Dissolve 69.28 g copper sulphate ($CuSO_4.5H_2O$) in distilled water and dilute to 1000 ml. Filter and store in amber colour bottle.

Fehling's solution B: Dissolve 346 g Rochelle salt (Potassium sodium tartrate: $KNa\ C_4H_4O_6.\ 4H_2O$) and 100 g NaOH in distilled water. Dilute to 1000 ml. Filter and store in amber colour bottle.

Neutral lead acetate solution: Prepare 20% neutral lead acetate solution.

Potassium oxalate solution: Prepare 10% potassium oxalate ($K_2C_2O_4.\ H_2O$) solution.

Methylene blue indicator: Prepare 1% methylene blue solution in distilled water.

4.2.3 Procedure

i) Standardization of the Fehling's Solution for Invert Sugar

Accurately weigh 4.75g of AR grade sucrose. Transfer to 500 ml volumetric flask with 50 ml distilled water. Add 5 ml conc. HCl and allow to stand for 24 hr. Neutralise the solution with NaOH using phenolphthalein as end point indicator and make up to volume. Mix well and transfer 25 ml to a 100 ml volmetric flask and make up to volume (1 ml = 2.5 mg of invert sugar). Transfer to a burette having an off-set tip and titrate against Fehling's solution as described below for sample.

Observations

Titre $= V_1$ = ------ ml

Calculations

$$\text{Factor for Fehling's solution (g of invert sugar)} = \frac{\text{Titre} \times 2.5}{1000}$$

$$= 0.0025 \times V_1 = \text{------- g}$$

ii) Determination of Reducing Sugars

Preparation of sample

Weigh accurately 10-50 g sample as such (juices, beverages etc.) or homogenized sample (jams, preserves etc.) and transfer to 500 ml volumetric flask. Add about 100 ml water and neutralize with NaOH solution to phenolphthalein end point. Add 10 ml neutral lead acetate solution, shake and let stand for 10 min. Add potassium oxalate solution in small amounts until there is no further precipitation. Make up to volume, mix the solution well and filter through Whatman No. 1 filter circle. Transfer the filtrate to a 50 ml burette having an off-set tip.

Preliminary titration: Pipette out 5 ml each of Fehling A and B solutions into 250 ml conical flask. Mix and add about 10 ml water and a few pumice stone or glass beads. Dispense the sugar solution from the burette. Heat the solution to boiling. Add 3 drops of methylene blue indicator. Continue the addition of the sugar solution drop wise until the blue colour disappears to a brick-red end point. (The concentration of the sample solution should be such that the titre value is between 15 to 50 ml). Maintain a total boiling peiod of 3 min. Note down the titre value.

Final titration: Pipette out 5 ml each of Fehling A and B solutions into a 250 ml conical flask. Add sample solution about 0.05 to 1.0 ml less than titre value of the preliminary titration. Heat the flask to boiling. Add 3 drops of methylene blue indicator. Complete the titration within 1 min by adding 2 to 3 drops of sugar solution at a time, until the indicator is decolourized. At the end point, the boiling liquid assumes the brick red colour. Note down the titre value. Perform the titration in duplicate and take the average.

Note: i) Preliminary titration must be finished within 3 min.
ii) Conical flask should not be disturbed or removed from the burner before the titration is finished.

4.2.4 Observations

Weight of the sample = W = ------- g

Dilution volume for the sample = V_2 = ------ ml

Volume of clarified sample solution required for Fehling's reaction (titre) = V_3 = ------- ml

4.2.5 Calculations

Based on the factor for Fehling's solution, V_3 ml sample solution contains:

0.0025 V_1 g reducing sugar (as invert sugar)

$$\text{Therefore, \% Reducing Sugars in the sample} = \frac{0.0025 \times V_1 \times V_2 \times 100}{V_3 \times W}$$

$$= \frac{0.25 \times V_1 \times V_2}{V_3 \times W} = X\ \%$$

4.2.6 Results

Reducing sugars (as invert sugar) = % by wt.

4.3 EXPERIMENT 4b: TOTAL REDUCING SUGARS

4.3.1 Principle

Total reducing sugars represent reducing sugars and non-reducing di and oligo saccharides, which can be hydrolysed into reducing sugars with dilute acids.

4.3.2 Requirements

Same as for experiment 4a.

4.3.3 Procedure

Pipette an aliquot of 50 ml of the clarified, de-leaded filtrate to a 100 ml volumetric flask. Add 5 ml of conc. HCl and allow to stand at room temperature for 24 hours. Neutralise with conc. NaOH solution followed by 0.1N NaOH using phenolphthalein as end point indicator. Make up to volume and transfer to 50 ml burette having an off-set tip. Perform the titration against Fehling's solution similar to the procedure described for reducing sugars, and determine the total sugars as invert sugars.

4.3.4 Observations

Volume of the acid hydrolysed sample solution required for Fehling solution (titre) = V_4 = ------ ml

4.3.5 Calculations

Based on the factor for Fehling's solution, total reducing sugars in

V_4 ml = 0.0025 × V_1 g

As 50 ml of the clarified and de-leaded solution is diluted twice (50 ml to 100 ml) after hydrolysis, dilution volume of the sample = ($2 \times V_2$).

$$\text{Therefore, \% Total reducing sugars (as invert sugars)} = \frac{0.0025 \times V_1 \times 2 \times V_2 \times 100}{V_4 \times W}$$

$$= \frac{0.5 \times V_1 \times V_2}{V_4 \times W} = Y\ \%$$

Total reducing sugars comprises of reducing sugars and non-reducing sugars, which can be hydrolysed into reducing sugars under the experimental conditions. This non-reducing sugar is usually expressed in terms of sucrose.

As 0.95 g sucrose on hydrolysis yields 1 g invert sugar (glucose + fructose):

% Sucrose in the sample = (Total reducing sugars – % Reducing sugars originally present) × 0.95

$$= (Y - X) \times 0.95$$

[% Total sugars = (% Reducing sugars + % Sucrose)]

4.3.6 Results

Sucrose content in the sample = % by weight

4.4 EXPERIMENT 4c: STARCH

4.4.1 Principle

Starch is hydrolysed to glucose with strong acid and the reducing sugar formed is estimated by Lane and Eynon method. Sample containing sugars are washed to free it from them before hydrolysing the starch. Traces of lipids present are also washed off with petroleum ether before hydrolysing the starch.

4.4.2 Requirements

500 ml conical flask with std. joint to fix Liebig condenser
Petroleum ether
Alcohol – 95%and 50%
10% Alpha naphthol solution in alcohol
Sulphuric acid- conc.
Stainless steel vessel (5 lit. cap.), 10” dia.
Test tube – 10 ml
Centrifuge

4.4.3 Procedure

For samples containing sugars and less starch, to weighed quantity (50-100 g), add a little of water and heat to 60°C. Allow to stand for some time to obtain a solution of starch. Add about 100 ml 95% alcohol and centrifuge (2000 rpm, 10 min.) to settle the precipitate. Filter and wash the residue with about 50% alcohol until the filtrate gives no positive test for sugars. To test for sugars, to a few ml of the filtrate in a test tube, add a drop of 10% alpha naphthol reagent. Allow 1 ml of pure conc. H_2SO_4 to flow slowly down the side of the test tube

so as to form a layer beneath the aqueous solution. If sugars are present, a red ring will appear within a few seconds at the junction of the two layers. Use the 50% alcohol-washed and dried precipitate for starch hydrolysis as below.

For starchy materials, weigh accurately about 2-5 g sample in a beaker. Add ether and stir well. Allow to settle and decant the ether portion and discard. Repeat the step 3-4 times and dry the sample.

Transfer the prepared sample to a conical flask and add 200 ml water and 20 ml conc. HCl. Place the flask in a steel vessel filled with 2/3rds volume of water. Connect the flask to a water-circulating condenser. Heat the vessel and allow the water to boil for exactly 2 hr. Shake the digestion flask intermittently during boiling. Remove the flask and cool the contents. Transfer the contents to a 500 ml volumetric flask and neutralise with sodium hydroxide. Filter through Whatman No. 1 filter circle. From the filtrate determine the reducing sugar content using Lane and Eynon titrimetric procedure.

4.4.4 Observations

Weight of sample	= W	= ------ g
Hydrolysed sample volume made up to:	= V_5	= ----- ml
Titre	= V_6	= ----- ml

4.4.5 Calculations

Based on the factor for Fehling's solution, reducing sugar content of V_6 ml of the hydrolysed starch solution = $0.0025 \times V_1$ g

The theoretical yield of reducing sugars (glucose) on complete hydrolysis of starch is: 0.90 g starch = 1 g glucose. However, for practical purposes, the currently accepted factor is: 0.925 g starch = 1 g glucose.

$$\text{Therefore, \% Starch content of the sample} = \frac{0.0025 \times V_1 \times V_5 \times 100 \times 0.925}{V_6 \times W}$$

4.4.6 Results

Starch content of the sample = per cent by weight.

4.5 PRECAUTIONS

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.

EXPERIMENT 5 DETERMINATION OF CRUDE FIBRE

Structure

5.1 INTRODUCTION

Crude fibre is the organic residue, which remains after the food sample has been treated with petroleum ether, boiling dilute sulphuric acid, dilute sodium hydroxide solution and alcohol under the standardized conditions. The crude fibre consists largely of cellulose together with a little lignin.

Objectives

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

- estimate the crude fibre content of food materials and products.

5.2 EXPERIMENT: DETERMINATION OF CRUDE FIBRE

5.2.1 Principle

By treating a food material successively with petroleum ether, sulphuric acid and sodium hydroxide, all the lipids, carbohydrates etc. are removed/ hydrolysed leaving only the crude fibre along with some insoluble mineral matter. The insoluble residue is freed of the soluble materials by water washing and filtration, and ashed. The difference in weight of the alcohol washed residue (dried) and the ash give the weight of true crude fibre.

5.2.2 Requirements

Equipment and Apparatus

Chemical balance, 1 mg sensitivity

Air oven (maintained at $100 \pm 2\ ^0C$)

Muffle furnace ($525 \pm 5°C$)

Hot plate

Digestion flask – 500ml

Water-jacketed condenser

Desiccator

Sintered glass crucible (porosity 100-160 μm) or

Linen cloth having 45 threads / inch.

Chemicals

0.255 N Sulphuric acid solution – 1.25 g H_2SO_4/100 ml

0.313 N Sodium hydroxide solution – 1.25 g NaOH/100 ml, free or nearly so from sodium carbonate.

Petroleum ether

Ethyl alcohol

5.2.3 Procedure

Grind the sample in a grinder to pass through No. 30 mesh sieve. Mix well to get a homogenous sample. Extract 2 g sample with ether and transfer residue to the digestion flask. Add 200ml hot sulphuric acid (1.25%) and connect to the reflex condenser and heat (it is essential that the solution boils within one minute). Boiling is continued briskly for exactly 30 min. Rotate flask frequently until sample at sides is thoroughly wetted. Take care to keep material from remaining on the sides of the flask. Immediately filter through linen cloth and wash with boiling water until the washings are acid free. Wash the residue back into the flask with 200ml hot sodium hydroxide solution (1.25%). Connect flask to reflux condenser and boil briskly exactly for 30 min. Remove the flask immediately and filter the contents through sintered crucible. Carefully transfer the entire residue to the flask with hot water. Wash the residue in the sintered crucible with hot water until the filtrate is alkali free. Wash with ethyl alcohol followed by ether. Then, dry at 100^0C to constant weight (W_1). Transfer the sintered crucible to a muffle furnace at 525^0C and ash the material. Cool and weigh (W_2). The loss in weight represents crude fibre.

5.2.4 Observations

Weight of the sample taken for ether extraction = W = ---- g

Weight of the sample after acid and alkali treatment along with sintered crucible = W_1 = -----g

Weight of residue after ashing along with the sintered crucible = W_2 ---- g

5.2.5 Calculations

Weight of crude fibre in the sample = Weight of acid and alkali digested residue minus weight of the ash = $W_1 - W_2$

$$\text{Crude fibre \%} = \frac{(W_1 - W_2) \times 100}{W}$$

$$\text{Crude fibre \% on dry wt.} = \frac{(W_1 - W_2) \times 100 \times 100}{W \times (100 - M)}$$

Where, M = % moisture content of the sample.

5.2.6 Results

Crude fibre = % by wt. on dry basis

5.3 PRECAUTIONS

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.

EXPERIMENT 6 DETERMINATION OF ALCOHOL BY SPECIFIC GRAVITY METHOD

Structure

6.1 INTRODUCTION

Alcohol content in alcoholic beverages like wines, beer, distilled spirits etc. is routinely determined. Good quality fruit juices contain practically no alcohol. The trace amount measured by distillation method is due to the other volatile components. However, if over ripe fruits are used for juice production or the juice has undergone some fermentation due to improper handling of the juice, the alcohol content will increase. Therefore, high alcohol content in fruit juices is an indication of poor quality.

Objectives

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

- carry out distillation of beverages to recover alcohol; and
- determine alcohol content by specific gravity method.

6.2 EXPERIMENT: DETERMINATION OF ALCOHOL

6.2.1 Principle

Alcohol present in fruit juices and beverages can be distilled out completely along with water. Based on the differences in specific gravities of water and alcohol, the alcohol content can be determined.

6.2.2 Requirements

Apparatus and Reagents

Alcohol distillation apparatus

Specific gravity bottle, 50 ml capacity

Electronic balance, 0.1 mg sensitivity

Volumetric flask

Thermostatically controlled water bath

Chemicals

Bromothymol blue indicator (1% solution)

0.1 N Sodium hydroxide solution

6.2.3 Procedure

Expel CO_2 from the sample if present by shaking thoroughly. Fill the sample in a 100 ml volumetric flask to just below the mark and immerse the flask in a water bath maintained at a constant temperature (usually 25^0C). After the sample has attained the bath temperature, make up the volume with more sample. Transfer the contents of the volumetric flask to the distillation flask (300 – 500 ml round bottomed flask) using 10 – 15 ml distilled water to rinse the flask thrice. Add a few drops of bromothymol blue indicator and titrate with 0.1 N NaOH to a distinctive blue colour to neutralize any volatile acids present. Connect the condenser to the distillation flash and let the tip of the condenser dip into about 10 ml of distilled water contained in the original 100 ml volumetric flask. Distil and collect the distillate almost to the 100 ml mark. Place volumetric flask in the water bath to attain the same temperature as was done for the sample and make up the volume with distilled water.

Weigh a specific gravity bottle accurately. Fill the bottle with the alcohol distillate, insert the stopper, remove the spill-over solution with a tissue paper and weigh. Transfer the solution back to the volumetric flask, clean and fill the bottle with distilled water and weigh as done for the sample. Calculate the specific gravity of the sample distillate and read the alcohol percentage from table of specific gravity vs. alcohol % by volume.

6.2.4 Observations

Weight of the empty sp. gravity bottle = W_1 = ----- g

------ do ---- with sample distillate = W_2 = ----- g

------ do ---- with distilled water = W_3 = ----- g

6.2.5 Calculations

Specific gravity of the sample distillate =

$$\frac{\text{Weight of a known volume of sample}}{\text{Weight of same volume of distilled water}} = \frac{(W_2 - W_1)}{(W_3 - W_1)}$$

Determine ethyl alcohol content (per cent by volume) corresponding to the specific gravity by using the following Table

6.2.6 Results

Alcohol content of sample = percent (v/v).

Ethyl alcohol content (v/v) at 15.56^{o}C (60^{o}F) corresponding to specific gravity

Apparent sp.gr	Alcohol conc. %	Apparent sp.gr	Alcohol conc. %
0.9992	0.53	0.9813	14.05
0.9990	0.66	0.9801	15.06
0.9985	1.00	0.9790	16.00
0.9976	1.60	0.9778	17.04
0.9970	2.01	0.9767	18.00
0.9955	3.04	0.9755	19.06
0.9941	4.02	0.9744	20.03
0.9927	5.02	0.9622	30.02
0.9913	6.06	0.9472	40.05
0.9900	7.05	0.9288	50.04
0.9887	8.06	0.9075	60.03
0.9875	9.01	0.8837	70.02
0.9862	10.05	0.9572	80.02
0.9850	11.00	0.8270	90.02
0.9837	12.06	0.7981	100.00
0.9825	13.04		

6.3 PRECAUTIONS

The general precautions mentioned in the course ‘Introduction’ and those indicated in the experiments should be followed meticulously.

Avoid holding the body of the specific gravity bottle, especially after filling solutions. This will cause expansion of the solution, which will flow out resulting in wrong results. Hold the bottle at the neck.

EXPERIMENT 7 DETECTION AND DETERMINATION OF SYNTHETIC COLOURS

Structure

7.1 INTRODUCTION

Only eight synthetic coal-tar dyes are permitted under PFA for addition to food products. PFA also specifies the foods to which such colours should not be added as well as the maximum permissible limits (0.2 g per kg) of the colours in the foods in which the colours are permitted.

Among the food additives, synthetic colours are viewed with extreme caution due to their potential toxicity to the human system. Therefore, identification and quantification of synthetic food colours in foods is very important.

Objectives

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

- identify coal-tar dyes added to foods; and
- determine the concentration of the colour(s) in the food product.

7.2 EXPERIMENT

7.2.1 Principle

Synthetic acidic colour(s) is dyed on to wool in acidic medium and extracted (stripped) from the wool into aqueous alkaline medium. The extracted colour(s) is developed (separated) by paper chromatography along with standard dyes using a suitable solvent system. Comparing their Rf values with that of standard colours identifies the sample colours. Quantification of the colours is done by spectrophotometry.

7.2.2 Requirements

Instruments and Apparatus

Chemical balance

Spectrophotometer with 1 cm quarts cells

Measuring cylinders – 25 ml, 50 ml

Volumetric flasks – 50 ml, 100 ml

Conical flasks – 100 ml, 250 ml

Beakers – 100 ml

Wool

Chromatographic chamber

Thin layer chromatogrphic plates

Chromatography column – glass column with tapered end (2.1 × 45 cm) filled with alumina

Glass capillaries

Chemicals

Butanol: Acetic acid: Water (BAW) solvent: 20: 5: 12 (v/v/v)

Liquor ammonia

Acetic acid

Sodium citrate

pH paper strips

Alumina (acidic aluminium oxide)

Standard reference colours

White knitting wool: Successively boiled in dilute ammonia, washed in water, boiled in dilute acetic acid, again washed in water and dried.

Whatman No. 1 and No.3 Chromatographic paper, 20 × 20 cm

7.2.3 Procedure

I. Detection

1. *Wool dyeing:* Take 50 ml or 50 g of the sample, add enough distilled water and prepare a free-flowing solution. Add 4 to5 pieces of 5 cm long woollen thread to the solution and acidify the solution with acetic acid (few ml, check with pH paper) and boil for 10 to 20 minutes. Take out the pieces of wool from the solution and wash in water. Transfer the woollen pieces to a beaker and strip (extract) the colour by boiling with dilute ammonia (1 part of liquor ammonia + 50 parts of distilled water) and remove the wool. Make the extracted solution acidic with acetic acid. Immerse fresh small pieces of wool in the extract and boil for 10 min. If the wool is not dyed then report absence of added artificial colouring matter. If the wool is dyed, it indicates the presence of a coal-tar dye. Wash the wool with water and again strip the colour in boiling ammonia solution, filter; evaporate to a small volume in a beaker for chromatography. The above method is not suited for basic dyes.

   For basic dyes reverse the method i.e. dye the wool first in dilute ammonia and then strip in acetic acid. At present, all the permitted water-soluble coal-tar dyes are acidic and hence an indication of the

presence of basic dye at this stage indicates presence of unpermitted colour.

2. *Paper chromatography:* Draw a horizontal line 2.5 cm from base of the filter paper (Whatman No.1). Spot the extracted colour solution on the line along with standard colour (dye) solutions and develop the chromatogram using one of the most effective solvent system viz. "BAW" – Butanol : Acetic acid : Water (20:5:12). When solvent front runs to a height of about 15 cm, remove the chromatogram and dry. Compare the sample Rf with the standard Rf, and identify the colour.

$$\text{Rf value} = \frac{\text{Distance moved by the solute (colour)}}{\text{Distance moved by the mobile phase (solvent)}}$$

Standard colour spot(s) corresponding to the sample colour(s) = --------
name of the standard colour(s)

II. Quantification

Samples containing single colour

Weigh about 5-10 g sample. Add about 25 ml water and mix well. Pass the solution through a column containing acidic aluminium oxide. Wash the column with water to remove sugars and natural colours. Elute the adsorbed colour with 1% ammonia. Transfer the eluate to a volumetric flask (25 ml) and make up to volume with 0.1 N HCl. Determine the absorbance of the dye solution at the absorption maxima (λ max).

Samples containing mixture of colours

Elute the mixture of colours by column chromatography and make up to a known volume (5 to 10 ml). Streak an aliquot of 0.5 ml on Whatman No. 3 paper and develop the chromatogram using the solvent system. Dry the paper and cut out the individual colour bands and elute with 0.1 N HCl. Make up to a known volume and determine the absorbance of each of the dye solutions at their absorption maxima.

7.2.4 Observations

a) Sample containing single colour

Weight of the sample	= W = ------ g
Volume of column eluate made up	= V = ------ ml
Absorbance of the solution	= A ----------
$E^{1\%}_{1cm}$ of the dye at λ max	= E ----------

b) Sample containing mixture of colours

Weight of the sample	= W_1 = ------ g
Volume of column eluate made up	= V_1 = ------- ml
Volume of the eluate streaked on chromatographic paper	= V_2 = ----- ml
Volume of made up HCl extract of each colour band	= V_3 = ----- ml
Absorbance of the made up HCl extracts	= A1, A2, A3 ---- An
$E^{1\%}_{1cm}$ of the dye	= E1, E2, E3 ----- En

7.2.5 Calculations

a) Dye content of the product (%) = $\dfrac{\text{Absorbance} \times \text{Volume of eluate made up} \times 100}{E^{1\%}_{1cm} \text{ of the dye} \times 100 \times \text{Wt. of sample}}$

$$= \frac{A \times V}{E \times W}$$

Therefore, dye content of the product (ppm) = $\dfrac{A \times V}{E \times W} \times 10{,}000$

b) Calculate the content of each dye separately. Here, calculation for the dye having absorbance = A1 and $E^{1\%}_{1cm}$ = E1 is given as an example.

Content of the dye in the product (ppm) = $\dfrac{A \times V_3 \times V_1 \times 10{,}000}{E \times V_2 \times W_1}$

$E^{1\%}_{1cm}$ for standard permitted food colours are given below.

$E^{1\%}_{1cm}$ = Extinction (Absorbance) of 1 % solution of a dye at its absorption maxima when measured in a 1 cm cell.

Colour	λ_{max} (nm)	$E^{1\%}_{1cm}$
Tartrazine	426	527
Sunset Yellow FCF	480	551
Ponceau 4R	505	431
Carmoisine	515	545
Erythrosine	526	1154
Indigo carmine	610	489
Fast Green FCF	625	1560
Brilliant Blue FCF	629	1637

7.2.6 Results

The quantity of added colour is expressed in parts per million (ppm) or mg/kg.

7.3 PRECAUTIONS

The general precautions mentioned in the course 'Introduction' and those indicated in the experiments should be followed meticulously.

While handling the dye solutions care should be taken to prevent it from getting them into the mouth because some of them may be unpermitted and harmful.

The spectrophotometer is a very costly instrument. Use it only after carefully understanding its operation. Similarly, the silica cells used are very costly and brittle. Handle them with utmost care. The silica cells should be wiped only with soft tissue to prevent scratching the transparent glass surface.