UNIT 1 CLASSIFICATION OF MICROORGANISMS IMPORTANT IN THE FOOD INDUSTRY: BACTERIA, YEASTS AND MOLD

Structure

1.0 OBJECTIVES

The objectives of this unit are to enable you to understand the important genera of microorganisms associated with food. This unit gives a brief account of the morphological, physiological and cultural characteristics of various microorganisms. After going through this unit, you should be able to:

- know the various types of microorganisms;
- explain the requirements for their growth;
- learn the classification of these organisms based on their characteristics; and
- distinguish between the useful and harmful microorganisms.

1.1 INTRODUCTION

We already know that the microorganisms use our food as a source of nutrients for their own growth. This, of course can result in deterioration of the food. By increasing their numbers, utilizing nutrients, producing enzymatic changes and contributing off flavors by means of breakdown of a product or synthesis of new compounds they can “spoil” a food. When the microorganisms involved are pathogenic, their association with our food supply is critical from a public health point of view. Therefore a classification of different organisms and their growth requirements is required to prevent spoilage of foods.

1.2 VARIOUS TYPES OF MICROORGANISMS

Microbes are single-cell organisms so tiny that millions can fit into the eye of a needle. They are the oldest form of life on earth. Microbe fossils date back more than 3.5 billion years to a time when the Earth was covered with oceans that regularly reached the boiling point, hundreds of millions of years before

dinosaurs roamed the earth. Without microbes, we couldn't eat or breathe. Without us, they'd probably be just fine.

Bacteria

Many of us know bacteria only as "germs," invisible to naked eyes that can invade our bodies and make us sick. Few know that many bacteria not only coexist with us all the time, but help us do an amazing array of useful things like make vitamins, break down garbage, and even maintain our atmosphere. These are unicellular microorganisms that are classed as plants. A bacterial cell is about 1μm in length and somewhat smaller in diameter. Bacteria are classified according to their shape. Cocci are spherical, bacilli are cylindrical and spirilla and vibrios are spiral. Bacterial spores are more heat resistant than yeast or mold spores to most processing conditions. Bacteria, with a few exceptions cannot grow in acid media in which yeasts and molds thrive. They multiply by 'binary fission'. When a bacterium becomes mature it divides into two, these two become four and so on. Bacteria can be found virtually everywhere. They are in the air, the soil, and water, and in and on plants and animals, including us. A single teaspoon of topsoil contains about a billion bacterial cells (and about 120,000 fungal cells and some 25,000 algal cells). The human mouth is home to more than 500 species of bacteria. Some bacteria (along with archaea) thrive in the most forbidding, uninviting places on Earth, from nearly-boiling hot springs to super-chilled Antarctic lakes buried under sheets of ice. Microbes that dwell in these extreme habitats are aptly called extremophiles. The growth of bacteria is very rapid and depends upon the nature of the food material, moisture, temperature and air. Some bacteria do not grow in air but temperature plays a major role in their growth, the optimum being generally 37°C for bacteria pathogenic to humans.

Bacteria are very sensitive to acids and are destroyed in their presence even at temperature of boiling water. Hence, most fruits being acidic can be easily sterilized at 100°C whereas vegetables being non-acidic require a higher temperature of 116°C.

A bacterium's genetic information is contained in a single DNA molecule suspended in a jelly-like substance called cytoplasm. In most cases, this and other cell parts are surrounded by a flexible cytoplasmic membrane that is itself surrounded by a tough, rigid cell wall. A few species, such as the mycoplasmas, don't have cell walls.

Even though bacteria have only one cell each, they come in a wide range of shapes, sizes, and colours.

The important groups of bacteria are:

a) Bacillus: rod-shaped.

b) Coccus: spherical.

c) Coccobacillus: oval-shaped.

d) Aerobes: require atmospheric oxygen for growth, e.g., *Acetobacter aceti*.

e) Facultative anaerobes: can grow with or without atmospheric oxygen.

f) Obligate anaerobes: do not grow in atmospheric oxygen.

g) Mesophiles: require a temperature below 38°C for growth.

h) Obligate thermophiles: grow between 38°C and 82°C.

i) Facultative thermophiles: grow over a wide range of temperatures covered by mesophiles and obligate thermophiles and below.

j) Psychrotrophs: grow fairly well at refrigeration temperatures and some can even grow slowly at temperatures below freezing.

Some bacteria have natural colours. Certain species contain pigments, such as various chlorophylls, that make them naturally green, yellow, orange, or brown. Colonies of millions of bacteria may appear pink, yellowish, or white.

Important Food Spoilage Bacteria

Group	Genus
Acetics	*Acetobacter* and *Gluconobacter*
Lactics	*Lactobacillus, Leuconostoc, Pediococuus, Sreptococcus*
Butyrics	*Clostridium*
Propionics	*Propionibacterium*
Proteolytics	*Bacillus, Pseudomonas, Closridium, Proteus etc.*

Some useful bacteria

The following bacteria are of great importance in the food processing industry.

Acetobacter sp.

These bacteria, also known as “vinegar bacteria”, cause significant spoilage in the wine industry but are necessary for vinegar production. The important species are *Acetobacter aceti, A. orleansis and A.schutzenbachi*. They are very small, usually non-motile and generally do not form spores. These bacteria are aerobes and in the presence of oxygen convert ethyl alcohol to acetic acid. These bacteria can be easily destroyed by heating to 65°C.

Lactobacillus sp.

Different organisms of this group, also known as “lactic acid bacteria”, have different properties but all of them produce lactic acid from carbohydrates. The important species include *Lactobacillus plantarum, Pediococcus cerevisiae, Leuconostoc mesenteroides, Streptococcus faecalis* and *Lactobacillus brevis.* These bacteria cause “lactic souring” and spoil wines, which can be easily prevented by maintaining a sulphur dioxide concentration of 0.007 per cent in wine.

Yeasts

Yeasts are unicellular fungi which are widely distributed in nature. They are somewhat larger than bacteria. The cell length is about 10μm and the diameter is about a third of this. Most yeasts are spherical or ellipsoidal. Yeasts that multiply by means of ‘budding’ are known as ‘true yeasts’. Yeasts grow luxuriously at a moderate temperature in a solution of sugar in plenty of water.

Under suitable conditions the sugar is converted into alcohol and carbon dioxide is evolved. This is the reason that carbon dioxide is evolved from food materials spoiled by yeasts and pushes out corks from bottles with great force. Most of them do not develop in media containing more than 66% sugar or 0.5% acetic acid. Boiling destroys the yeast cells and spores completely. Some of the yeasts which grow on fruits are *Saccharomyces, Candida* and *Brettanomyces.*

Pseudo-yeasts

These are like true yeasts but do not form spores. All the members of this group are particularly unsuitable for fermentation purposes as they produce off-flavours and cloudiness.

Yeasts causing food spoilage

Yeast	Product Spoilage
Saccharomyces	Low sugar products
Candida	High-acid foods, salty foods, butter
Brettanomyces	Beers, wines
Zygosaccharomyces (osmophillic)	Honey, syrups, molasses, wines, soy sauce
Pichia	Wines
Hansenula	Beers
Torulopsis	Milk products, fruit juices, acid foods
Rhodotorula	Meat, sauerkraut

Fungi (Molds)

Fungi are eukaryotic organisms. This means that their DNA-containing chromosomes are enclosed within a nucleus inside their cells. (The chromosomes of bacteria and archaea are not walled off inside nuclei, making them prokaryotic organisms). Molds are multicellular, filamentous fungi which are devoid of chlorophyll. They are larger than yeasts. They are strict aerobes and require oxygen for growth and multiplication and tend to grow more slowly than bacteria.

Fungi are lower thallophytic plants but do not make their own food via photosynthesis like green plants. They feed on organic matter like rotting leaves, wood, and other debris, or upon the tissues of living plants and animals.

Fungi, along with bacteria, are the planet's major composters and recyclers. Although fungi may seem like a nuisance when they grow in your fruit bowl or refrigerator, their ability to degrade some of the toughest organic materials, including tree wood and insect exoskeletons, means that our planet is not cluttered with a mass of debris. Fungi secrete digestive enzymes in order to break down complex food sources, such as animal corpses and tree stumps, into smaller components they can absorb.

The principle parts of a mold are a web-like structure known as mycelium and the spore. The mycelium is often white and cottony and penetrates into the

attacked foodstuff. After fixing itself the mold produces viable spores which resist the favourable conditions after the dispersal and germinate when they get favourable conditions. They thrive best in closed, damp and dark situations with an adequate supply or warm, moist air but require less free moisture than yeasts and bacteria. They prefer sugar containing substances and may spoil jams, jellies and other sugar-based products. Acid medium favours their growth and, therefore, they grow well in pickles, juices etc. this is the main reason that fruit and fruit products are attacked by molds which not only consume nutrients present in the food thereby lowering its food value but also spoil the flavour, texture and appearance of the product. Molds are sensitive to heat; boiling quickly destroys molds and their spores. The most important molds are:

a) *Penicillium* sp. (Blue moulds)
b) *Aspergillus* sp. (Black moulds)
c) *Mucor* sp. (Gray moulds)
d) *Bysslchlamyces fulva*

Classification of Microorganisms

A) On basis of temperature for growth

Microorganisms can be classified into:

- Thermophillic: Microbes who require high temperature for their growth and survival (optimum temperature=45-65ºC).
- Thermoduric: Microbes which do not grow at high temperatures but can survive in it.
- Mesophillic: Microorganisms which require optimum temperature of 20-50ºC for growth and multiplication.
- Psychrophillic: Microorganisms requiring less than 20ºC as optimal temperature for growth.
- Psychroduric: Microorganisms which do not grow at low temperature but can survive.

B) On basis of oxygen requirement for growth:

- Obligate Aerobes: Require oxygen for growth and multiplication e.g. molds.
- Obligate Anaerobes: Strictly grow only in absence of oxygen.
- Facultative: Microorganisms than can grow in both presence and absence of oxygen e.g. yeasts.
- Microaerophillic: Organisms which are able to grow at very low oxidation-reduction potential.

C) On basis of requirement of water activity.

In general, bacteria require more moisture than yeasts and yeasts more than molds.

The classification according to requirement of a_w is as follows:

Group of microorganism	Minimal a_w value
Bacteria	0.91
Yeast	0.88
Molds	0.80
Halophillic bacteria	0.75
Xerophillic fungi	0.65
Osmophillic Yeasts	0.60

- Halophillic bacteria: Bacteria which grow in high salt solutions
- Osmophillic Yeasts: Yeasts which can grow best in high concentrations of sugar
- Xerophillic Fungi: Fungi which can grow in low water activity

D) On basis of nutrient degradation capacity:

- Proteolytic: Microorganisms which are capable of protein degradation because of extracellular proteinases produced.
- Lipolytic: Microbes which catalyxe the hydrolysis of fats to fatty acids and glycerol.
- Sacchrolytic: These microorganisms hydrolyse disaccharides or polysaccharides to simpler sugars.
- Pectinolytic: These microorganisms hydrolyse pectin.

E) On basis of staining:

On basis of staining the bacteria can be classified as:

- Gram positive: Those bacteria that stain violet after Gram stain test. In these the cell wall is mostly comprised of peptidoglycan layer.
- Gram negative: Those bacteria that do not stain violet after Gram stain test. Cell wall mainly comprised of lipopolysaccharides.

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. Classify bacteria according to their morphology.

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2. Differentiate between yeasts and molds.

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3. Classify microorganisms on basis of the temperature requirements, oxygen requirements, water activity requirement, staining procedure and nutrient degrading capability.

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1.3 CHARACTERISTICS (MORPHOLOGICAL, CULTURAL AND PHYSIOLOGICAL) OF MICROORGANISMS

1.3.1 Bacteria

Morphological Characteristics

One of the first step in the identification of bacteria in food is microscopic examination to ascertain the shape, size, aggregation, structure and staining

reactions of the bacteria present. The following characteristics may be of special significance:

Encapsulation: The presence of capsules or slime may account for sliminess or ropiness of a food. Most capsules are polysaccharides of dextrin, dextran or levan and they serve as a source of reserve nutrients and increase the resistance of bacteria under adverse conditions.

Formation of Endospores: Bacteria of genera *Bacillus, Clostridium, Sporosarcina* etc have the ability to form endospores. Endospores are formed at an intracellular site and are resistant to heat, ultraviolet light and dessication. Lysis of the vegetative cell releases the free endospore, which may remain dormant with no detectable metabolism for years. Sporulation usually appears in the late logarithmic phase of growth, possibly because of nutrient depletion or product accumulation. The acquisition of heat resistance is closely related to the formation of dipicolinic acid and the Ca^{2+} uptake. Germination is favoured by conditions that are favourable for growth.

Formation of Cell Aggregates: It is characteristic of some bacteria to form long chains or of others to clump under certain conditions. It is more difficult to kill all bacteria in intertwined chains or sizable clumps than to destroy separate cells.

Cultural Characteristics

Bacterial growth in and on foods often is extensive enough to make the food unattractive in appearance or otherwise objectionable. Pigmented bacteria cause discolouration on the surfaces of foods; films which may cover the surfaces of liquids; growth may make surfaces slimy; or growth throughout the liquids may result in undesirable cloudiness or sediment.

Physiological Characteristics

Most bacteria may be placed into one of three groups based on their response to gaseous oxygen. Aerobic bacteria thrive in the presence of oxygen and require it for their continued growth and existence. Other bacteria are anaerobic, and cannot tolerate gaseous oxygen, such as those bacteria which live in deep underwater sediments, or those which cause bacterial food poisoning. The third group are the facultative anaerobes, which prefer growing in the presence of oxygen, but can continue to grow without it.

Bacteria may also be classified both by the mode by which they obtain their energy. Classified by the source of their energy, bacteria fall into two categories: heterotrophs and autotrophs. Heterotrophs derive energy from breaking down complex organic compounds that they must take in from the environment – this includes saprobic bacteria found in decaying material, as well as those that rely on fermentation or respiration.

The other group, the autotrophs, fix carbon dioxide to make their own food source; this may be fueled by light energy (photoautotrophic), or by oxidation of nitrogen, sulfur, or other elements (chemoautotrophic). While chemoautotrophs are uncommon, photoautotrophs are common and quite diverse. They include the cyanobacteria, green sulfur bacteria, purple sulfur bacteria, and purple nonsulfur bacteria. The sulfur bacteria are particularly interesting, since they use hydrogen sulfide as hydrogen donor, instead of water like most other photosynthetic organisms, including cyanobacteria.

Microbe is a term for tiny creatures that individually are too small to be seen with the unaided eye. Microbes include bacteria (*back-tear-ee-uh*), archaea (*are-key-uh*), fungi (*fun-jeye*) and protists (*pro-tists*). You've probably heard of bacteria and fungi before. Archaea are bacteria-like creatures that have some traits not found in any true bacteria. Protists include primitive algae (*al-gee*), amoebas (*ah-me-buhs*), slime molds and protozoa (*pro-toe-zoh-uh*). We can also include viruses (*vye-rus-is*) as a major type of microbe, though there is a debate as to whether viruses can be considered living creatures or not.

1.3.2 Molds

General Characteristics

The term "mold" is a common one applied to certain multicellular, filamentous fungi whose growth on foods usually is readily recognized by its fuzzy or cottony appearance. The main part of the growth commonly appears white but may be coloured or dark or smoky. Coloured spores are typical of mature mold of some kinds and give colour to part or all of the growth. The thallus, or vegetative body, is characteristic of thallophytes, which lack true roots, stems and leaves.

Morpohological Chactacteristics

The morphology, i.e. the form and structure, of molds, as judged by their macroscopic and microscopic appearance, is used in their identification and classification.

Hyphae and Mycelium: The mold thallus consists of a mass of branched, intertwined filaments called hyphae (singular hypha), and the whole mass of these hyphae are known as the mycelium.

Reproductive Parts or Structures: Molds can grow from a transplanted piece of mycelium. Reproduction of molds is chiefly by means of asexual spores. Some molds also form sexual spores.

Culture Characteristics

The gross appearance of a mold growing on a food often is sufficient to indicate its class or order. Some molds are loose and fluffy; others are compact. Some look velvety on the upper surface, some dry and powdery, and others wet or gelatinous. Some molds are restricted in size, while others seem limited only by the food or container. Pigments in the mycelium – red, purple, yellow, brown, gray black, etc. – are characteristic, as are the pigments of mass of asexual spores; green, blue-green, yellow, orange, pink, lavender, brown, gray, black, etc.

Physiological Characteristics

The physiological characteristics of molds will be reviewed only briefly here and will be discussed in more detail subsequently.

Moisture Requirements: In general most molds require less available moisture than do most yeasts and bacteria. It has been claimed that below 14 to 15 percent total moisture in flour or some dried fruits will prevent or greatly delay mold growth.

Temperature Requirements: Most molds would be considered mesophilic i.e. able to grow well at ordinary temperature. The optimal temperature for most

molds is around 25 to 30°C, but some grow well at 35 to 37°C or above, e.g. *Aspergillus* spp. And some at still higher temperatures. A number of molds are psychrotophic or psychroduric i.e. they grow fairly well at temperatures of refrigeration, and some can grow slowly at temperatures below freezing. Growth has been reported at as low as – 5 to 10°C. A few are thermophilic; i.e. they have a high optimal temperature.

Oxygen and pH Requirements Molds are aerobic; i.e. they require oxygen for growth; this is true at least for the molds growing on foods. Most molds can grow over a wide range of hydrogen-ion concentration (pH 2 to 8.5), but the majority are favoured by an acid pH.

Food Requirements: Molds in general can utilize many kinds of foods, ranging from simple to complex. Most of the common molds possess a variety of hydrolytic enzymes, and some are grown for their amylases, pectinases, proteinases, and lipases.

Inhibitors: Compounds inhibitory to other organisms are produced by some molds, such as penicillin from *Penicillium chrysogenum* and clavacin from *Aspergillus clavatus.* Certain chemical compounds are mycostatic, inhibiting the growth of molds (sorbic acid, propionates, and acetates are examples), or are specifically fungicidal, killing molds.

Initiation of growth of molds is slow compared to that of bacteria or yeasts, so that when conditions are favourable for all these organisms, molds usually lose out in the competition. After mold growth is under way, however, it may be very rapid.

1.3.3 Yeasts

Like mold, the term “yeast” is commonly used but hard to define. As used here it refers to those fungi which are generally not filamentous but unicellular and ovoid or spheroid and which reproduce by budding or fission.

Yeasts may be useful or harmful in foods. Yeast fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar, and surface-ripened cheese, and yeasts are grown for enzymes and for food. Yeasts are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups, molasses, honey, jellies, meats, wine, beer, and other foods.

Morphological Characteristics

Form and structure: The form of yeasts may be spherical to ovoid, lemon-shaped, pear-shaped, cylindrical, triangular, or even elongated into a false or true mycelium. They also differ in size.

Reproduction: Most yeasts reproduce asexually by multilateral or polar budding, a process in which some of the protoplasm bulges out the cell wall; the bulge grows in size and finally walls off as a new yeast cell. A new species or yeasts reproduce by fission, and one reproduces by combination of fission and budding.

Sexual reproduction of “true” yeasts *(Ascomycotina)* results in the production of ascospores, the yeast cell serving as the ascus. The ascospores may differ in colour, in smoothness or roughness of their walls, and in their shape (round, oval, reniform, bean or sickle-shaped, hemispherical, angular, fusiform, or needle-shaped).

"False" yeasts, which produce no ascospores or other sexual spores, belong to the *Fungi Imperfecti.* Cells of some yeasts become chlamydospores by formation of a thick wall about the cell, for example, *Candida, Rhodotorula, and Cryptococcus.*

Cultural Characteristics

For the most part, the appearance of massed yeast growth is not useful in the identification of yeasts, although growth as a film on the surface of liquid media suggests an oxidative or film yeasts, and production of a carotenoids pigment indicates the genus *Rhodotorula.* However, the appearance of the growth is important when it causes coloured spots on foods.

Yeasts are oxidative, fermentative, or both. The oxidative yeasts may grow as a film, pellicle, or scum on the surface of liquid and then are termed *film yeasts.* Fermentative yeasts usually grow throughout the liquid and produce carbon dioxide.

Physiological Characteristics

Most common yeasts grow best with a plentiful supply of available moisture. But since many yeasts grow in the presence of greater concentration of solutes (such as sugar or salt) than most bacteria it can be concluded that these yeasts require less moisture than the majority of bacteria. Most yeast require more moisture than molds, however, on the basis of water activity or aw yeasts may be classified as ordinary if they do not grow in high concentrations of solutes, i.e. in a low aw, and as osmophilic if they do. However limits of aw for ordinary yeasts tested thus far ranges from 0.88 to 0.94.

The range of temperature for growth of most yeasts is, in general, similar to that for molds, with the optimum around 25°C to 30°C and the maximum about 35°C to 47°C. Some kinds can grow at 0°C or less. The growth of most yeasts if favoured by an acid reaction in the vicinity of pH 4 to 4.5, and they will not grow well in an alkaline medium unless adapted to it. Yeasts grow best under aerobic conditions, but the fermentative types can grow anaerobically, although slowly.

In general, sugars are the best source of energy for yeasts, although oxidative yeasts, e.g., the film yeasts, oxidize organic acids and alcohol. Carbon dioxide produced by bread yeasts accomplishes the leavening of bread, and alcohol made by the fermentative yeasts is the main product in the manufacture of wines, beer, industrial alcohol, and other products. The yeasts also aid in the production of flavors or "bouquet" in wines.

Nitrogenous foods utilized vary from simple compounds such as ammonia and urea to amino acids and polypeptides. In addition, yeasts require accessory growth factors.

Microorganisms, namely, bacteria, yeasts and molds can be found in any environment. The eight environmental sources of organisms to foods are: soil and water, plants and plant products, food utensils, intestinal tracts of humans and animals, food handlers, animal feeds, animal hides, air and dust. Although we see that the microorganisms are beneficial to the humans in many ways, there are many microorganisms that are the causative agents for food borne diseases. e.g. *Staphlococcus aureus* and *Clostridium botulinum* cause food borne intoxication whereas *Salmonella, E.coli, Campylobacter, Listeria,*

Yersinia, Bacillus etc cause food borne infections. Molds are responsible for causing food intoxication by production of mycotoxins, which are lethal for the human body e.g. Aflatoxin produced by *Aspergillus flavus*, patulin produced by *Penicillium expansum*, ochratoxins produced by *Aspergillus ochraceus* etc. All these will be discussed in Unit 3.

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 formation of special structures by bacteria.

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2. What are the physiological requirements of molds?

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3. Yeasts maybe useful or harmful. Explain.

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1.4 LET US SUM UP

This unit briefly outlines the identification and classification of food microorganisms. After reading this unit, you will be able to classify microorganisms broadly into three categories: bacteria, yeasts and molds. Further you will be able to categorize these microorganisms on the basis of their requirements of temperature, oxygen, water activity for growth and also on the basis of their ability to degrade certain nutrients. To prevent the spoilage

of food products, appropriate measures have to be taken for preventing their growth and multiplication. Hence food microbiologists have to be well versed with the various morphological, cultural and physiological characteristics of the different microorganisms so as to prevent their growth and proliferation.

After reading this unit, you will get a knowledge of the factors that favour or inhibit the growth of microorganisms which are essential to give an understanding of the principles of food spoilage and preservation.

1.5 KEY WORDS

Bacteria : Unicellular microorganisms 1µm in length, lacking chlorophyll and multiply by binary fission.

Yeasts : Unicellular fungi, larger than bacteria, which multiply by budding.

Molds : Multicellular, filamentous fungi devoid of chlorophyll.

Endospore : Heat resistant structures formed by bacteria under unfavourable conditions (nutrient depletion or product accumulation) which remain dormant till exposed to favourable environment.

Capsule : Slimy material composed of polysaccharides that serve as a source of reserve nutrients and make cell resistant to adverse conditions.

Mycostatic : Substances which are inhibitory to mold growth.

Fungicidal : Substances capable of killing the fungi.

Thermophillic organisms : Microorganisms which require high optimum temperature for their growth and multiplication (45-60ºC).

Mesophillic organisms : Microorganisms whose optimum temperature for growth is 25-40ºC.

Proteolytic microorganisms : Microorganisms that have the capacity to break down complex proteins to amino acids due to production of extracellular protein degrading enzymes.

Gram positive bacteria : Bacteria that stain violet after Gram staining.

Gram negative bacteria : Bacteria that stain red after Gram staining.

Psychrophillic microorganisms : Microorganisms requiring less than 20ºC as optimal temperature for growth.

Obligate aerobes : Microorganisms that grow and multiply only in presence of oxygen.

Obligate anaerobes : Microorganisms that grow and multiply only in absence of oxygen.

Facultative microorganisms : Those microorganisms that can grow and survive under both aerobic and anaerobic conditions.

Microaerophillic microorganisms : Those microorganisms that can grow and survive under low concentrations of oxygen.

Thermoduric microorganisms : Those microorganisms that can survive at high temperatures but grows in mesophillic range.

1.6 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. On basis of morphology bacteria can be classified as:
   a) Bacilli: rod-shaped
   b) Coccus: spherical
   c) Coccobacillus: oval-shaped
   d) Spirilla: spiral shaped
2. Yeasts are unicellular fungi and are smaller in size whereas molds are multicellular and filamentous and are larger than yeasts. Yeasts multiply asexually by budding whereas molds reproduce sexually by spores. Yeasts can grow in anaerobic conditions but molds are strictly aerobic.
3. Classification of microorganisms on basis of temperature requirement:
   a) Thermophillic(45-60ºC)
   b) Thermoduric
   c) Mesophillic (25-40ºC)
   d) Psychroduric
   e) Psychrophillic

   Classification of microorganisms on basis of oxygen requirement:
   a) Obligate aerobes: Strictly grow in presence of oxygen
   b) Obligate anaerobes: Strictly grow in absence of oxygen
   c) Facultative: Can grow both in presence and absence of oxygen
   d) Microaerophillic: Grow at low oxygen concentrations

Classification of microorganisms on basis of a_w requirement:

a) Bacteria (a_w =0.9)
b) Yeast (0.88)
c) Mold (0.80)
d) Halophillic bacteria (0.75)
e) Xerphillic molds (0.65)
f) Osmophillic yeasts (0.60)

Classification of microorganisms on basis of nutrient degradation:

a) Lipolytic: Fat degrading
b) Saccharolytic: Sugar degrading
c) Pectinolytic: Pectin degrading
d) Proteolytic: Protein degrading

Classification of microorganisms on basis of staining:

a) Gram positive bacteria: Stain violet after staining
b) Gram negative bacteria: Stain red after Gram staining

Check Your Progress Exercise 2

1. Bacteria form special structures such as endospores, capsules and cell aggregates to combat the adverse environmental conditions. Bacteria of genera *Bacillus, Clostridium, Sporosarcina* etc have the ability to form heat resistant endospores. Sporulation usually appears in the late logarithmic phase of growth, possibly because of nutrient depletion or accumulation of toxic products. Endospores may remain dormant with no detectable metabolism for years and germinate when exposed to favourable conditions for growth. Capsules serve as a source of reserve nutrients and increase the resistance of bacteria under adverse conditions. They are composed of dextran and levan and account for sliminess or ropiness of a food. Bacteria may also form aggregates which helps them to combat unfavourable environments.

2. Physiological requirements of molds: Molds are mesophillic and grow well at 25-30°C. Some are psychrotrophic and can grow at –5 to 10°C. They are aerobic in nature and can grow at wide range of pH 2-8.5. They require a minimum moisture range of 14-15 per cent to grow. Owing to a number of hydrolytic enzymes, molds can proliferate on complex media also.

3. Yeasts may be useful or harmful in foods. Yeast fermentations are involved in the manufacture of foods such as bread, beer, wines, vinegar, and surface-ripened cheese, and yeasts are grown for enzymes and for food. Yeasts are undesirable when they cause spoilage of sauerkraut, fruit juices, syrups, molasses, honey, jellies, meats, wine, beer, and other foods.

1.7 SOME USEFUL BOOKS

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UNIT 2 FACTORS AFFECTING GROWTH AND INHIBITION OF MICROORGANISMS IN FOOD

Structure

2.0 OBJECTIVES

After reading this unit you should be able to:

- list out the various factors that favour/inhibit the growth of microorganisms;
- explain the role played by pH in inhibition of microbial growth;
- explain the effect of water activity on microbial growth and activities;
- explain the influence of redox potential on the natural microflora of food and the type of spoilage occurring in food;
- understand the role played by nutrient composition on type of microorganisms growing in food;
- understand the role played by antimicrobial barriers in retarding microbial spoilage of food; and
- understand the role played by inhibitory substances in retarding microbial spoilage of food.

2.1 INTRODUCTION

Microorganisms use our food supply as a source of nutrients and energy. They increase their numbers by utilizing nutrients. This can result in a deterioration of the food. They produce enzymatic changes and off-flavours in food by breaking down a nutrient or synthesizing new compounds. Thus, they "spoil" our food and make it unfit for consumption. To prevent this we reduce the contact between microorganisms and our foods (prevent contamination) and also eliminate microorganisms from our foods, or adjust conditions of storage in such a way that their growth is prevented (preservation) and thus, there is no spoilage of food.

If the microorganisms involved are pathogenic, then their presence in our food will lead to outbreak of food borne diseases also. Many of our foods support the growth of pathogenic microorganisms or serve as a source of them. Here again, we attempt to prevent their entrance and growth in our foods or eliminate them by processing.

Interactions between microorganisms and our foods are also beneficial. Many of the cultured products consumed and enjoyed for example cultured buttermilk, yoghurt, sauerkraut, pickles and tofu are produced as a result of beneficial activities of microorganisms.

Food is the substrate for growth of microorganisms, so the characteristics of a food are important. Food or substrate will determine which microorganisms can or cannot grow on it so there is a need to understand the characteristics of the food or substrate. Then only one can make predictions about the microbial flora that may develop and flourish in it. This microflora will bring about the biochemical changes in food due to their activities. The types of biochemical changes will determine whether those changes are beneficial or harmful.

Knowledge of the factors that favour or inhibit the growth of microorganisms is very important. It will help us in understanding the principles of food spoilage and preservation. The chief compositional factors of food that influence microbial activity are hydrogen-ion concentration, moisture, oxidation-reduction (O-R) potential, nutrients, biological structure and presence of inhibitory substances.

2.2 HYDROGEN-ION CONCENTRATION (pH)

The acidity and alkalinity (pH) of an environment has a strong influence on the activity and stability of macromolecules such as enzymes. These enzymes play an important role during growth of microorganisms and in their metabolism. Thus, growth and metabolism of microorganisms are influenced by pH.

2.2.1 Effect on Microbial Growth

Every microorganism has a minimal, a maximal, and an optimal pH for growth. In general, bacteria grow in the pH range of 6.0–8.0, yeasts 4.5-6.0 and filamentous fungi 3.5-4.0. Molds can grow over a wider range of pH than most yeasts and bacteria, and many molds grow at acidities too high for yeasts and bacteria. Most fermentative yeasts grow well in pH range of about 4.0 to 4.5, eg. fruit juices, and film yeasts grow well on acid foods, such as sauerkraut and pickles. On the other hand, most yeast do not grow well in alkaline foods and thus do not have a significant role to play in the spoilage of food products with high pH. However, large number of yeasts grow well in near neutral pH. There are some exceptions for example some bacteria can grow in moderate acidity particularly those bacteria that produce large acids as

a result of their activities like lactobacilli and acetic acid bacteria. These have pH optima between 5.0 and 6.0 and others like the proteolytic bacteria can grow in foods with a high (alkaline) pH, as found in the stored egg white. Bacteria are more sensitive to pH than molds and yeasts, with the pathogenic bacteria being the most sensitive amongst them. The pH values of some of the common foods along with the pH range for growth of some groups of microorganisms and a few of food associated pathogenic bacteria are given in Table 2.1.

Table 2.1: The pH ranges of some common food items and pH range of some common food microflora

Food	pH range	Organism	pH range
Citrus fruits	2-5	Molds	0-11
Soft drinks	2.5-4	Yeasts	1.5-8.5
Beer	3.5-4.5	Lactic acid bacteria	3.2-10.5
Meat	5.5-6.2	*Staphylococcus aureus*	4-9.8
Fish	6.5-7.3	*Salmonella* spp.	4.1-9
Egg white	8.6-9.6	*Escherichia coli*	4.3-9
Milk	6.5-7	*Yersinia enterocolitica*	4.5-9
Flour	6.2-7.2	*Clostridium botulinum*	4.8-8.2
Vegetables	4.8-7	*Clostridium perfringens*	5.4-8.7
Fermented shark	10-12	*Bacillus cereus*	4.7-9.3

pH minima and maxima of microorganisms also varies due to other important factors like temperature, moisture content, salt concentration, redox potential etc. For example, in the presence of 0.2 M NaCl, *Alcaligenes faecalis* can grow over a wider pH range than in the absence of NaCI or in the presence of 0.2 M sodium citrate. The pH minima of certain lactobacilli also depends upon the type of acid used, for example with citric, hydrochloric, phosphoric and tartaric acids growth can occur at lower pH than in presence of acetic or lactic acids. In general, yeast and molds are more acid-tolerant than bacteria.

When microorganisms are grown at pH either higher or lower than their optimum pH there is an increase in lag phase of the microbe. The increased lag would be of longer duration if the food has a good buffering capacity in contrast to one that has poor buffering capacity. Good buffering capacity of food would result in slower change in pH of food due to microbial activity. A respiring microbial cell is adversely affected by pH since it affects the functioning of enzymes and the transport of nutrients into the cell. In addition to the effect of pH on rate of growth of microorganisms, pH also affects rate of survival of microorganisms during storage, heating, drying and other forms of processing. Many times the initial pH may be suitable, but growth of the organism itself may alter the pH, thereby making it unfavourable. Conversely, the initial pH may be restrictive, but the growth of a limited number of microorganisms may alter the pH to a more favourable range for the growth of many other microorganisms.

The inherent pH of foods varies, although most are neutral or acidic. Materials with an alkaline pH generally have a rather unpleasant taste with some exceptions like egg white where the pH increases to around 9.2, as CO_2 is lost from the egg after laying. The pH of a product can be easily determined with a

pH meter. However, this value alone is not sufficient for predicting microbial spoilages. It is also desirable, for example, to know the acid responsible for a given pH, because some acids, particularly the organic acids, are more inhibitory than others.

2.2.2 Effect on Microbial Ecology and Food Spoilage

The acidity of a product plays an important role in deciding the type microflora present in food and the rate and type of its spoilage. For example, most of the meats and seafoods have a final ultimate pH of about 5.6 and above. Thus, these products are susceptible to bacterial as well as to mold and yeast spoilage. Similarly, most vegetables have higher pH values than fruits, and thus vegetables would be more prone to bacterial than fungal spoilage since such pH values favour bacterial growth. Soft-rot producing bacteria such as *Erwinia carotovora* and pseudomonads play a significant role in their spoilage. In fruits, however, a lower pH (below 4.5) prevents bacterial growth and yeasts and molds dominate spoilage.

Fish is spoiled more rapidly than meat under chilled conditions. This is due to the fact that the pH of post-rigor mammalian muscle is around 5.6 and this contributes to the longer storage life of meat. On the other hand, fish have a pH between 6.2-6.5. *Shewanella* (formerly *Alteromonas*) mainly causes spoilage under chilled conditions. It is a pH-sensitive microbe and hence, plays a significant role in fish spoilage but not in normal meat (pH<6.0). Those fishes that have a naturally low pH such as halibut (pH~5.6) as a result have better keeping qualities than other fish. Thus, a food with inherently low pH would tend to be more stable microbiologically than a neutral food.

Upon the death of a well-rested meat animal, the usual 1% glycogen is converted into lactic acid, which directly causes a depression in pH values from about 7.4 to about 5.6. Most of the bacteria cannot tolerate lower pH, hence meat has a longer storage life. Meat from fatigued animals spoils faster than that from rested animals. This is because most of the glycogen present had already been used during its lifetime and hence, final pH attained upon completion of rigor mortis is not as low as that of a well-rested animal. Thus, bacteria are able to grow and spoil it.

The excellent keeping quality of certain foods is related to their restrictive pH, for example fruits, soft drinks, fermented milks, sauerkraut and pickles which have an acidic pH. Fruits, soft drinks, vinegar, and wines have an excellent keeping quality mainly due to pH, which falls far below the point at which bacteria normally grow. Fruits generally undergo mold and yeast spoilage, and this is due to the capacity of these organisms to grow at pH values < 3.5, which is considerably below the minima for most food spoilage and all food poisoning bacteria.

Some foods have a low pH because of inherent acidity; others, for example, the fermented products like sauerkraut, pickles and fermented milks have a low pH because of acidity produced due to the activity of microorganisms. This acidity is also known as biological acidity and is generally due to the accumulation of lactic acid during fermentation. Regardless of the source of acidity, the effect upon keeping quality appears to be the same. This ability of low pH to restrict microbial growth has been employed since the earliest times for preservation of foods using acetic acid and lactic acids.

2.2.3 Inhibition of Microbes by Weak Acids

With the exception of those soft drinks that contain phosphoric acid, in most

other acidic foods acidity is due to the presence of weak organic acids. These do not dissociate completely into protons and conjugate base in solution but establish equilibrium:

$$HA \rightleftharpoons H^+ + A^-$$

The partial dissociation of weak acids, such as acetic acid, plays an important role in their ability to inhibit microbial growth. Although addition of strong acids has a more profound effect on pH but at the same pH, they are less inhibitory than weak lipophilic acids. This is because microbial inhibition by weak acids is directly related to the concentration of undissociated acid (Figure 2.1). These undissociated lipophilic acid molecules can pass freely through the membrane, in doing so they pass from an external environment of low pH where the equilibrium favours the undissociated molecule to the high pH of the cytoplasm. At this higher pH, the equilibrium shifts in favour of the dissociated molecule, so the acid ionizes producing protons. These protons tend to acidify the cytoplasm. The cell tends to maintain its internal pH by expelling protons leaking in. This process requires energy and the microbe diverts energy from growth related functions to removing protons from the cell thereby slowing its growth. The burden on the cell becomes too great. The cytoplasmic pH drops to a level where growth is no longer possible and the cell eventually dies. Strong acids on the other hand dissociate completely into protons and conjugate base in solution. These dissociated acid molecules cannot pass freely through the cell membrane. Hence there is not much change in the pH of the cytoplasm. As a result these are less inhibitory than weak acids at the same pH.

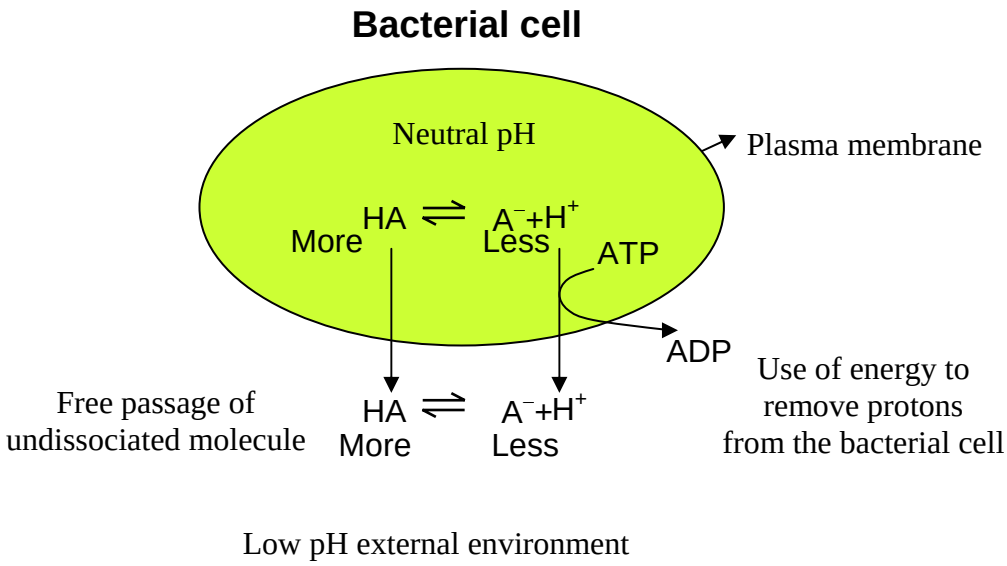

Figure 2.1: Inhibition of bacterial growth by weak acids

2.2.4 Buffers in Foods

Some foods are better able to resist changes in pH than others. These tend to resist changes in pH since these are buffered and the ability to resist changes in pH is known as buffering capacity. The buffers are the compounds present in food that resist changes in pH and thus are important. These are especially effective within a certain pH range. Buffers permit an acid (or alkaline) fermentation to go on longer with a greater yield of products and organisms

than would otherwise be possible. In general, meats are more buffered than vegetables. Contributing to the buffering capacity of meats are their various proteins. Vegetables are generally low in proteins and consequently lack the buffering capacity to resist changes in their pH by the growth of microorganisms. Hence these permit an appreciable decrease in pH with the production of small amounts of acid by the lactic acid bacteria during the early part of sauerkraut and pickle fermentations. This is desirable since it enables the lactic acid bacteria to suppress the undesirable pectin-hydrolyzing and proteolytic organisms which cause spoilage. Low buffering power makes for a more rapidly appearing succession of microorganisms during fermentation than high buffering power. Milk is fairly high in protein (a good buffer) and therefore permits considerable growth and acid production by lactic acid bacteria during the manufacture of fermented milks before growth is suppressed.

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 do fishes spoil more rapidly than meat?

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2. Why does the meat from fatigued animal spoil faster than that from a well-rested animal?

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3. What is biological acidity?

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4. Why are the weak organic acids more inhibitory to growth of microorganisms than the strong acids?

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5. How does adverse pH affect the microorganism?

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2.3 MOISTURE REQUIREMENT/WATER ACTIVITY

One of man's oldest methods of preserving foods is drying or desiccation. The preservation of foods by drying is a direct result of removal of moisture. Microorganisms need water for growth. Without water no growth can occur. The exact amount of water needed for growth of microorganisms varies. This water requirement of microorganisms is best expressed in terms of available water or water activity a_w, the vapour pressure of the solution (of solutes in water in most foods) divided by the vapour pressure of the solvent (usually water). Thus a_w, for pure water would be 1.00. The water activity depends on the number of molecules and ions present in solution, rather than their size. Thus a compound like sodium chloride, which dissociates into two ions in solution, is more effective at reducing the water activity than a compound like sucrose on mole-to-mole basis.

2.3.1 Effect on Microbial Growth and Activity

Bacteria require higher values of a_w for growth than fungi. Gram-negative bacteria have higher requirements than gram positives. Most spoilage bacteria do not grow below a_w 0.91, while spoilage molds can grow as low as 0.80. However, food-poisoning bacteria like *Staphylococcus aureus* can grow at a_w as low as 0.86, while *Clostridium botulinum* does not grow below 0.94. Yeasts and molds can grow over a wider a_w range than bacteria. The lowest a_w values for bacteria is 0.75 for halophilic (meaning salt-loving) bacteria, while

xerophilic (dry-loving) molds and osmophilic (preferring high osmotic pressures) yeasts can grow at a_w values of 0.65 and 0.60, respectively. The limiting value of water activity for the growth of microorganisms is about 0.6 and below this value the spoilage of foods is not due to microorganisms but may be due to insect damage or chemical reaction such as oxidation. At a water activity of 0.6, corresponding to a water potential of -68MPa (Mega Pascals), the cytoplasm would need to contain very high concentrations of an appropriate compatible solute and it is probable that the macromolecules such as DNA would no longer function properly and active growth may stop.

Most bacteria grow well in a medium with a water activity a_w approaching 1.00 (at 0.995 to 0.998), i.e., they grow best in low concentrations of sugar or salt. Culture media for most bacteria contain not more than 1 per cent of sugar and 0.85 per cent of sodium chloride (physiological salt solution). As little as 3 to 4 percent sugar and 1 to 2 percent salt may inhibit some bacteria. The optimal a_w and the lower limit of a_w for growth vary with the bacterium, as well as with food, temperature, pH, and the presence of oxygen, carbon dioxide, and inhibitors. The optimal a_w and the lower limit of a_w for growth is lower for bacteria which are able to grow in high concentrations of sugar or salt. Some examples of lower limits of a_w for growth of some food bacteria are given in Table 2.2. These figures would vary depending on conditions used for growth of the microorganisms as mentioned above.

Table 2.2: Minimum a_w values for growth of microorganisms of importance in food

Organisms Groups	Water activity (a_w)	Organisms Specific organisms	Water activity (a_w)
Most spoilage bacteria	0.90	*Pseudomonas* spp.	0.97
Most spoilage yeasts	0.88	*Escherichia coli*	0.96
Most spoilage molds	0.80	*Bacillus subtilis*	0.95
Halophilic bacteria	0.75	*Enterobacter aerogenes*	0.945
Xerophilic molds	0.61	*Clostridium botulinum*	0.93
Osmophilic yeasts	0.60	*Staphylococcus aureus*	0.86

Molds differ considerably in optimal a_w and range of a_w for the germination of asexual spores. The minimal a_w for spore germination is as low as 0.62 for some molds and as high as 0.93 for others (e.g., *Mucor*, *Rhizopus*, and *Botrytis).* Each mold also has an optimal a_w and range of a_w for growth. Examples of optimal a_w are 0.98 for *Aspergillus* sp., 0.995 to 0.98 for *Rhizopus* sp., and 0.9935 for *Penicillium* sp. The a_w would have to be below 0.62 to stop all chances for mold growth, although a_w below 0.70 inhibits most molds that cause food spoilage. The reduction of the a_w below the optimum for a mold delays spore germination and reduces the rate of growth and therefore is an important factor in food preservation. Many of the molds can grow in foods with a_w approaching 1.00 (pure water).

With a reduction of water activity of food, the number of microorganisms

capable of maintaining active growth in it decreases. On the other hand, there are microorganisms that grow better at reduced a_w. These microorganisms are generally associated with foods having low water activity. Since low water activities are associated with three distinct types of food, the following three terms are used to describe the microorganisms especially associated with these foods:

i) **Halotolerant** – able to grow in the presence of high concentrations of salt

ii) **Osmotolerant** – able to grow in the presence of high concentrations of nonionized organic compounds such as sugars.

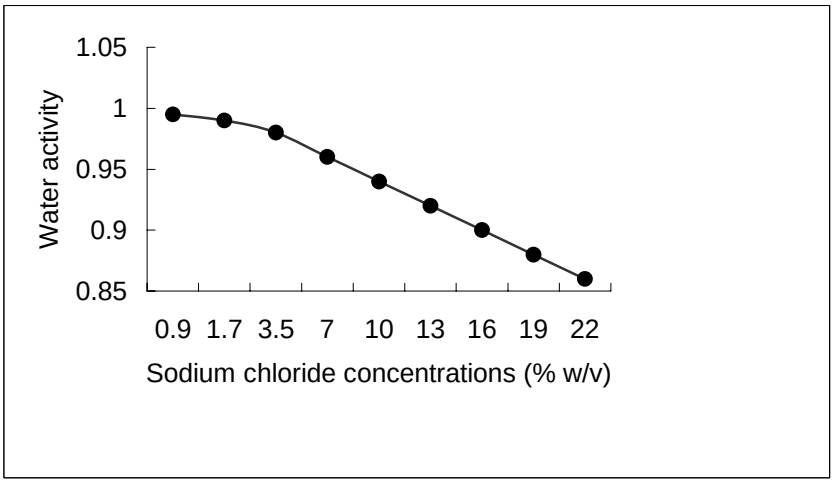
iii) **Xerotolerant** – able to grow on dry foods.

The halobacteria are obligately halophilic and cannot grow in the absence of high concentration of salt.

2.3.2 Ways of Reducing Water Activity

Water is made unavailable in various ways:

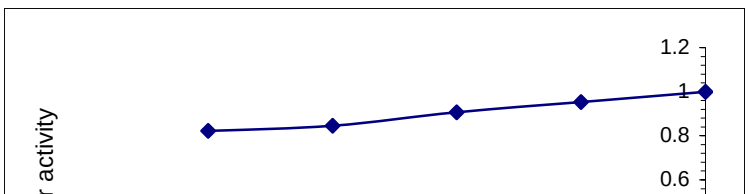
1. *Solutes and ions tie up water in solution*. Solutes lower a_w and this reduction in a_w depends on the total concentration of dissolved molecules and ions. Since these bind to water molecules, there is reduction in a_w. Therefore, an increase in the concentration of dissolved substances such as sugars and salts is in effect a drying of the material (Graph 2.1). Not only is water tied up by solutes, but also water tends to leave the microbial cells by reverse osmosis to maintain equilibrium between the concentration of solute outside and inside the cells.



Graph 2.1: Effect of sodium chloride concentration on water activity

2. *Hydrophilic colloids (gels) make water unavailable*. As little as 3 to 4 percent agar in a medium may prevent bacterial growth by leaving too little available moisture.

3. *Water of crystallization or hydration is usually unavailable to microorganisms*. Water itself, when crystallized as ice, no longer can be used by microbial cells. The a_w of water-ice mixtures (vapour pressure of ice divided by vapour pressure of water) decreases with a decrease in temperature below 0^oC (Graph 2.2). In a food, as more and more ice is formed, the concentration of solutes in the unfrozen water increases, thus lowering available water and thereby it's a_w is reduced.



Graph 2.2: Effect of temperature on water activity

The water activity a_w varies with temperature; these variations are only slight within the range of temperatures that permit microbial growth. Variations in temperature increase in importance with increasing concentrations of solutes and increasing effects on ionization of solutes.

Each microorganism has a maximal, optimal, and minimal a_w for growth. As the a_w is reduced below the optimal level, there is a lengthening of the lag period of growth, a decrease in the rate of growth and a decrease in the amount of cell substance synthesized, changes that vary with the organism and with the solute employed to reduce a_w. This range depends on a number of factors which are mentioned below.

2.3.3 Factors Affecting Water Requirement

Factors that may affect a_w requirements of microorganisms include:

1. *Kind of solute employed to reduce the a_w*: For some organisms, like molds, the lowest a_w for growth is independent of the kind of solute used. For other organisms, however, lower limiting a_w values differ from solute to solute. For example potassium chloride usually is less toxic than sodium chloride, and it in turn is less inhibitory than sodium sulphate. Thus, sodium sulphate at a lower concentration may be as effective in reducing a_w as potassium chloride at a higher concentration.

2. *Nutritive value of the culture medium*: In general, the better the medium for growth, the lower the limiting a_w permitting growth of microorganism.

3. *Temperature*: Most organisms have the greatest tolerance to low a_w at about optimal temperatures.

4. *Oxygen supply*: Growth of aerobes takes place at a lower a_w in the presence of air than in its absence, and the reverse is true of anaerobes.

5. *pH*: Most organisms are more tolerant of low a_w at pH values near neutrality than in acid or alkaline media.

6. *Inhibitors*: The presence of inhibitors narrows the range of a_w for growth of microorganism.

Each organism has its own characteristic optimal a_w and its own range of a_w for growth in a given set of environmental conditions. This range of a_w permitting growth is narrowed if any of the above mentioned environmental factors are not optimal and is narrowed still more if two or more conditions are not favourable. An unfavourable a_w will result not only in a reduction in the rate of growth but will also reduce the yield of cells. The delay (lag) in initiation of growth or germination of spores will be more under more unfavourable a_w of the substrate. It is known that growth of at least some cells

may occur in high numbers at reduced a_w values, but the production of certain extracellular products may be limited or these may not be produced at all. For example, reduced a_w results in the cessation of enterotoxin B production by *Staphylococcus aureus* even though high numbers of cells are produced at the same time This often is as important in food preservation as reduction in the rate of growth of the organism. Microorganisms that can grow in high concentrations of solutes, e.g., sugar and salt, obviously have a low minimal a_w. Halophilic bacteria require a certain minimal concentration of dissolved sodium chloride for growth. Osmophilic yeasts grow best in high concentrations of sugar.

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 out the types of microorganisms associated with foods having low water activity.

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2. Define water activity.

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3. How water is made unavailable to microorganisms?

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4. Describe the factors that may affect a_w requirements of microorganisms.

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2.4 OXIDATION REDUCTION POTENTIAL

The tendency of a substrate to accept or donate electrons, is termed its redox potential (*E*h). The O/R potential of a substrate may be defined generally as the ease with which the substrate loses or gains electrons. When a substrate loses electrons, the substrate is oxidized while a substrate that gains electrons becomes reduced. Therefore, a substance that readily gives up electrons is a good reducing agent, while one that readily takes up electrons is a good oxidizing agent. In the equation below, this is represented in its most general form to include the many redox reactions, which also involve protons and have the overall effect of transferring hydrogen atoms.

$$[\text{Oxidant}]+H^{+}+ne \rightleftharpoons [\text{Reductant}]$$

Where *n* is the number of electrons, e, transferred.

The tendency of an atom or molecule to accept or donate electrons is expressed as its standard redox potential, *E*o'. When electrons are transferred from one compound to another, a potential difference is created between the two compounds. This difference may be measured by use of an appropriate instrument and expressed as millivolts (mv). It can be measured against an external reference by an inert metal electrode, usually platinum. The more highly oxidized a substance, the more positive will be its electrical potential, and the more highly reduced a substance, the more negative will be its electrical potential.

2.4.1 Redox Couples in Food

Pair of oxidizing and reducing agents present in food are known as redox couples. A large positive *E*o' of food indicates that the oxidized species of the couple is a strong oxidizing agent and the reduced form only weakly reducing. A large negative *E*o' of food indicates the reverse. When the concentration of oxidant and reductant is equal, a zero electrical potential exists. The relative proportions of oxidized and reduced species present will also influence the measured Eh. If the balances of the various redox couples present favours the oxidized state then there will be a tendency to accept electrons from the electrode creating a positive potential, which signifies an oxidizing-environment. If the balance is reversed, the sample will tend to donate electrons to the electrode, which will then register a negative potential – a reducing environment.

With the notable exception of oxygen, most of the couples present in foods, *e.g.* glutathione and cysteine in meats and ascorbic acid and reducing sugars in plant products, would on their own tend to establish reducing conditions. Oxygen, which is present in the air at a level of around 21 %, is usually the most influential redox couple in food systems. It has a high *E*o' and is a powerful oxidizing agent. If sufficient air is present in food, a high positive potential will result and most other redox couples present will, if allowed to equilibrate, be largely in the oxidized state. Hence, increasing the access of air to food material by chopping, grinding or mincing will increase its *E*h. Similarly, exclusion of air as in modified vacuum packing or canning will reduce the *E*h.

2.4.2 Effect of Microbial Growth on Redox Potential of Food

Microbial growth in food reduces its *E*h. This is usually because during their growth, microorganisms consume oxygen and produce reducing compounds such as hydrogen. Oxygen is the most important terminal electron accepter in the electron transport chain, especially in case of aerobes. During passage of electrons through the electron transport chain, microorganisms generate energy and thereby oxygen is depleted. As the oxygen content of the medium decreases, so the redox potential declines from a positive potential to a negative potential.

The decrease in *E*h as a result of microbial activity is the basis of some rapid tests for determination of microbial load of food, particularly dairy products. Redox dyes such as methylene blue or resazurin are used to indicate changes in *E*h, which are correlated with microbial levels. These dyes become colourless when these are reduced. The time taken for reduction of the dyes will be inversely proportional to the microbial load of food i.e. more the microorganisms in food, less is the time taken for dye to be reduced and *vice versa*. The factors influencing redox potential of foods are summarized in Table 2.3 given below:

Table: 2.3 Factors affecting redox potential of foods

1. Redox couples present
2. Ratio of oxidizing species to reducing species
3. pH
4. Poising capacity
4. Availability of oxygen
5. Microbial activity

2.4.3 Effect on Microbial Growth and Ecology

Redox potential exerts an important selective effect on the microflora of a food since it will decide the type of microorganism which can grow in that food. Microbial growth can occur over a wide spectrum of redox potential. However, individual microorganisms have their own redox ranges over which they can grow. They are classified into one of several physiological groups on the basis of the redox range over which they can grow and their response to oxygen. Based on their ability to use free oxygen, microorganisms have been classified as

1. **Aerobic** when they require free oxygen.
2. **Anaerobic** when they grow best in the absence of free oxygen.
3. **Facultative** when they grow well either aerobically or anaerobically.

Molds are aerobic, most yeast grow best aerobically and bacteria may be aerobic, anaerobic, or facultative. A high (oxidizing) potential favours aerobes but will permit the growth of facultative organisms also, and a low (reducing) potential favours anaerobic or facultative organisms. Growth of an organism may alter the O-R potential of a food enough to inhibit other organisms. Anaerobes, for example, may lower the O-R potential to a level which is inhibitory to aerobes.

Obligate aerobes are those organisms that generate their energy from oxidative phosphorylation using oxygen as the terminal electron acceptor. Consequently, they have a requirement for oxygen and a high *E*h and will predominate at food surfaces exposed to air or where air is readily available, for example, pseudomonads, such as *Pseudomonas fluorescens,* which grows

at an *E*h of +100 to +500 mv, and other oxidative Gram-negative rods. These grow on meat surfaces and produce slime and off-odours. *Bacillus subtilis (E*h -100 to +135 mv) produces ropiness in the open texture of bread and *Acetobacter* species growing on the surface of alcoholic beverages oxidize ethanol to acetic acid to produce vinegar or spoil the alcoholic beverage.

Plant juices, tend to have *E*h values of +300 to +400 mv. It is not surprising to find that aerobic bacteria and molds are the common cause of spoilage of products of this type. Minced meats have *E*h values of around +200 mv while in solid meats the *E*h is generally around -200 mv. Cheeses have *E*h values on the negative side from –20 to around –200 mv.

Obligate anaerobes grow only at low or negative redox potentials and require absence of oxygen. Anaerobic metabolism gives the organism a lower yield of utilizable energy than aerobic respiration. A reducing environment minimizes the loss of reducing power from the microbial cell and thus, is favoured. Hence, presence of oxygen, which provides an oxidizing environment to the microbes is not favoured. However, for many anaerobes, oxygen itself exerts a specific toxic effect. For example, *Clostridium acetobutylicum* can grow at an *E*h as high as +370 mv maintained by ferricyanide, but would not grow at +110mv in an aerated culture. This effect is due to the inability of obligate anaerobes to scavenge and destroy toxic products of molecular oxygen such as hydrogen peroxide and superoxide anion radical (0_2^-) produced by one electron reduction of molecular oxygen. They lack the enzymes catalase and superoxide dismutase, which catalyse the breakdown of these radicals.

Thus, in a highly oxidized food, there will be a predominance of aerobic organisms especially at food surfaces exposed to air. Whereas, in food with negative *E*h, the anaerobic microflora requiring reduced conditions will be favoured. For example, anaerobic bacteria do not multiply until the onset of rigor mortis (stiffening of body after death) of muscles of horse because of the high *E*h (+250 mv) in prerigor meat. At 30 h postmortem (after death), the *E*h falls to about -130 mv in the absence of bacterial growth and this low *E*h values favour the growth of obligate anaerobes like *Clostridium.* Obligate anaerobes, such as clostridia, have the potential to grow wherever conditions are anaerobic such as deep in meat tissues and stews, in vacuum packs and canned foods causing spoilage and C. *botulinum* is of major public health concern, since it causes botulism.

Aerotolerant anaerobes are incapable of aerobic respiration, but can nevertheless grow in the presence of air. Many lactic acid bacteria fall into this category. They can only generate energy by fermentation and lack both catalase and superoxide dismutase, but are able to grow in the presence of oxygen because they have a mechanism for destroying superoxide.

Microorganisms affect the *E*h of their environment during growth. This is true especially of aerobes, which can lower the *E*h of their environment while anaerobes cannot. As aerobes grow, oxygen in the medium is depleted, resulting in the lowering of *E*h. Growth is not slowed, however, due to the ability of cells to make use of oxygen donating or hydrogen-accepting substances in the medium. The result of this is that the medium becomes poorer in oxidizing and richer in reducing substances. Microorganisms can reduce the *E*h of a medium by their production of certain metabolic by-products such as hydrogen sulphide, which has the capacity to lower *E*h to –300 mv. Since hydrogen sulphide reacts readily with oxygen, it will accumulate only in anaerobic environments.

2.4.4 Poising Capacity of Food

As redox conditions change, there will be some resistance to change in a food's redox potential. This is known as poising capacity of food. This capacity is dependent on the concentration of the redox couple. Poising is greatest when the two components of a redox couple are present in equal amounts.

Most fresh plant or animal foods have a low and well-poised O-R potential in their interior: the plants because of reducing substances such as ascorbic acid and reducing sugars and the animal tissues because of SH (sulfhydryl) and other reducing groups. As long as the plant or animal cells respire and remain active, they tend to poise the O-R system at a low level, resisting the effect of oxygen diffusing from the outside. Therefore, a piece of fresh meat or a fresh whole fruit would have aerobic conditions only at and near the surface. The meat could support aerobic growth of slime-forming or souring bacteria at the surface at the same time as anaerobic putrefaction could be proceeding in the interior.

Processing procedures may alter this situation. For example, heating may reduce the poising power of the food by destroying or altering the reducing and oxidizing substances present and also allow more rapid diffusion of oxygen inward, either because of the destruction of poising substances or because of changes in the physical structure of the food. Processing also may remove oxidizing or reducing substances. For example, clear fruit juices loose reducing substances by their removal during extraction and filtration and therefore become more favourable to the growth of yeasts than the original juice containing the pulp.

In the presence of limited amounts of oxygen the same aerobic or facultative organisms may produce incompletely oxidized products, such as organic acids, from carbohydrates, while with plenty of oxygen available, complete oxidation to carbon dioxide and water might result. Protein decomposition under anaerobic conditions may result in putrefaction, whereas under aerobic conditions, the products are likely to be less obnoxious. Thus, the redox potential of the food would decide the course of spoilage and the type of end products being produced due to microbial activities.

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. Define redox potential.

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2. Enlist the physiological groups of microorganisms based on their oxygen requirement.

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3. Define the poising capacity of food.

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4. How is the poising capacity of food destroyed?

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5. Enlist the factors which affect the redox potential of food.

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2.5 NUTRIENT CONTENT

Microorganisms use foods as a source of nutrients and energy. Each kind of microorganism has a definite range of food requirements. For some species that range is wide and growth takes place in a variety of substrates e.g. coliform bacteria; but others, e.g., many of the pathogens, being fastidious in their nutrient requirements can grow in limited kinds of substrates. The better the medium for an organism, the wider the ranges of temperature, pH, and a_w over which growth can take place.

The food based on their nutrient composition can be classified as (1) foods for energy, (2) foods for growth, and (3) accessory food substances, or vitamins, which may be necessary for energy or growth.

2.5.1 Foods for Energy

The carbohydrates, especially the sugars, are most commonly used as an energy source, but other carbon compounds may also serve the purpose, e.g., esters, alcohols, peptides, amino acids, organic acids and their salts. Comparatively few organisms can utilize complex carbohydrates, e.g., cellulose and starch. Microorganisms differ even in their ability to use some of the simpler soluble sugars. Many organisms cannot use the disaccharide lactose (milk sugar) and therefore do not grow well in milk. Some yeast do not attack maltose. Most organisms, if they utilize sugars at all, can use glucose. The ability of microorganisms to hydrolyze pectin, which is characteristic of some kinds of bacteria and many molds, is important in the softening or rotting of fruits and vegetables or fermented products got from them. The ability to synthesize amylolytic (starch degrading) enzymes will favour the growth of an organism on cereals and other starchy products. The addition of fruits containing sucrose and other sugars to yoghurt increases the range of carbohydrates available and allows the development of a more diverse spoilage microflora of yeasts.

Bacteria differ in their ability to utilize different foods as a source of energy. Some can use a variety of carbohydrates, e.g., the coliform bacteria and *Clostridium* spp., and others only one or two. Some can use other carbon compounds like organic acids and their salts, alcohols, and esters *(Pseudomonas* spp.). Some can hydrolyze complex carbohydrates, although others cannot.

A limited number of microorganisms can obtain their energy from fats but do so only if a more readily usable energy food, such as sugar, is absent. First, the fat must be hydrolyzed with the aid of lipase to glycerol and fatty acids, which then can serve as energy source for the hydrolyzing organism or others microbes that grow on products of lipid hydrolysis. Aerobic microorganisms are more commonly involved in the decomposition of fats than anaerobic ones, and the lipolytic organisms usually are also proteolytic.

Split products of proteins, for example, peptides and amino acids, serve as an energy source for many proteolytic organisms when a better energy source is lacking. These also serve as source of energy for some non-proteolytic organisms. Meats for example, may be low in carbohydrate and therefore will be decomposed by proteolytic species, e.g., *Pseudomonas* spp.

Molds in general can utilize many kinds of foods as energy source, ranging from simple to complex. Most of the common molds possess a variety of

hydrolytic enzymes and some are grown for their amylases, pectinases, proteinases, and lipases.

Not only is the kind of energy food important but also its concentration in solution and hence it's osmotic effect and the amount of available moisture, which will determine its growth rate. For a given percentage of sugar in solution, the osmotic pressure will vary with the weight of the sugar molecule, Therefore, a 10% solution of glucose has about twice the osmotic pressure of a 10% solution of sucrose or maltose; i.e., it ties up twice as much moisture. Molds can grow in the highest concentrations of sugars and yeasts in fairly high concentrations but most bacteria grow best in fairly low concentrations. There are, of course, some exceptions to this generalization: osmophilic yeasts grow in as high concentrations of sugar as molds and some bacteria can grow in fairly high concentrations of sugar.

An adequate supply of foods for growth will favour utilization of the foods for energy. More carbohydrate will be used if a good nitrogen food is present in sufficient quantity than if the nitrogen is in poor supply. Organisms requiring special accessory growth substances might be prevented from growing if one or more of these vitamins were lacking, and thus the whole course of decomposition might be altered due to a change in the microflora.

2.5.2 Foods for Growth

Microorganisms differ in their ability to use various nitrogenous compounds as a source of nitrogen for growth. The primary nitrogen sources utilized by heterotrophic microorganisms are amino acids. A large number of other nitrogenous compounds may serve this function for example, nucleotides, free amino acids, peptides and proteins. Simple compounds such as amino acids will be utilized by most of the organisms before they utilize complex compounds such as high molecular weight proteins. The nitrogen requirements of some bacteria such as *Pseudomonas* spp. may be satisfied by simple compounds like ammonia or nitrates whereas for others like lactics, more complex compounds like amino acids, peptides, or proteins may be utilized or even required.

Many molds are proteolytic, but comparatively few bacteria and very few yeast are actively proteolytic. Proteolytic bacteria grow best at pH values near neutrality and are inhibited by acidity. Only exceptions are the acid-proteolytic bacteria that hydrolyze protein while producing acid. Carbon for growth for most of the microorganisms is derived from organic compounds but some can use carbon dioxide also.

The minerals required by microorganisms are nearly always present at the low levels required.

2.5.3 Accessory Food Substances or Vitamins

Bacteria also vary in their need for vitamins or accessory growth factors. Some microorganisms are unable to synthesize some or all of the vitamins needed for their growth. For example, *Staphylococcus aureus* synthesizes part while *Pseudomonas* or *Escherichia coli* all of the factors needed. The lactics and many pathogens must have all of the vitamins furnished. Most natural plant and animal foodstuffs contain an array of these vitamins, but some may be low in amount or lacking. For example, meats are high in B vitamins and fruits are low, but fruits are high in ascorbic acid.

Microorganisms may require B vitamins in low quantities and most of the natural foods have an abundant quantity of these. Gram positive bacteria are the least synthetic and must, therefore, be supplied with one or more of these compounds before they will grow. Gram negative bacteria and molds are able to synthesize most of their requirements. Consequently, these two groups of organisms may be found growing on foods low in B vitamins. Fruits tend to be lower in B vitamins than meats. Thus, the usual spoilage of fruits is by molds rather than bacteria since fruits also have a low pH and positive *E*h, which favour mold growth.

Egg white contains biotin but also contains avidin, which ties it up, making it unavailable to microorganisms and thus eliminate spoilage of eggs through biotin requiring organisms. The processing of foods often reduces the vitamin content. For example, thiamine, pantothenic acid, folic acid and ascorbic acid (in air) are heat-labile. Drying causes a loss in vitamins such as thiamine and ascorbic acid. Even storage of foods for long periods, especially if the storage temperature is elevated, may result in a decrease in the level of some of these growth factors.

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. What is the effect of sugar concentration on microbial growth?

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2.6 BIOLOGICAL STRUCTURE

The plants and animals that serve as food sources have all evolved mechanisms of defense against the invasion and proliferation of microorganisms. By taking these natural phenomena into account, one can make effective use of these in preventing the microbial spoilage of the products.

2.6.1 Antimicrobial Barriers

The inner parts of whole, healthy tissues of living plants and animals are either sterile or low in microbial content. Therefore, unless opportunity has been given for their penetration, spoilage organisms within raw food may be few or lacking.

The first barrier is the integument: a physical barrier to protect the food, e.g., the shell on eggs, the skin on poultry, the shell on nuts and the rind or skin on fruits and vegetables, or these may be surrounded by natural wax. It is usually composed of macromolecules relatively resistant to degradation and provides an inhospitable environment for microorganisms either with a low water activity or nutrients deficiency or antimicrobial compounds e.g. short chain fatty acids on animal skin, essential oils on plant surfaces etc. This physical protection to the food may not only help in its preservation but may also determine the kind, rate and course of spoilage. Layers of fat over meat may protect that part of the flesh, or scales may protect the outer part of the fish.

2.6.2 Effect of Destruction of Microbial Barriers

Physical damage to the integument allows microbial invasion of the underlying nutrient-rich tissues and it is a common observation that damaged fruits and vegetables deteriorate more rapidly than entire products and that this process is initiated at the site of injury. Consequently, it is important that during harvesting and transport these barriers are maintained intact as far as possible.

An increase in exposed surface, brought about by peeling, skinning and chopping may serve not only to distribute spoilage organisms but also to release juices containing food materials for the microorganisms. The disintegration of tissues by freezing may accomplish a similar result. In meat the growth of spoilage bacteria takes place mostly in the fluid between the small meat fibers and it is only after rigor mortis that much of this food material is released from the fibers to become available to spoilage organisms.

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. What is the role of antimicrobial barriers in preventing food spoilage by microorganisms?

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2. What happens when integument is physically damaged?

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2.7 INHIBITORY SUBSTANCES

These may be originally present in the food, added purposely or accidentally, or developed there by growth of microorganisms or by processing methods. These may prevent growth of all microorganisms or, more often, may deter certain microorganisms. The mechanism of action for nearly all antimicrobials can be classified into one or more of the following groups: (1) reaction with the cell membrane, (2) inactivation of essential enzymes, or (3) destruction or functional inactivation of genetic material.

2.7.1 Biological Inhibitory Substances Originally Present in Food

The stability of some foods against attack by microorganisms is due to the presence of certain naturally occurring substances e.g. plants such as mustard, horseradish, watercress, cabbage and other brassicas produce antimicrobial isothiocyanates (mustard oils) (Fig. 2.2) and in *Allium* species (garlic, onions and leeks) thiosulfinates such as allicin. Antimicrobials collectively known as phytoalexins are produced by many plants in response to microbial invasion, for example phaseollin an antifungal compound is produced in green beans.
Many natural constituents of plant tissues such as pigments, alkaloids and resins also have antimicrobial properties. Benzoic and sorbic acids found in cranberries and mountain ash berries respectively are commonly used in their pure forms as food preservatives. The anthocyanins are a group of water-soluble pigments, which occur naturally in fruits. The aglycone portion of these compounds, the anthocyanidins, has antimicrobial powers against several bacterial spp.

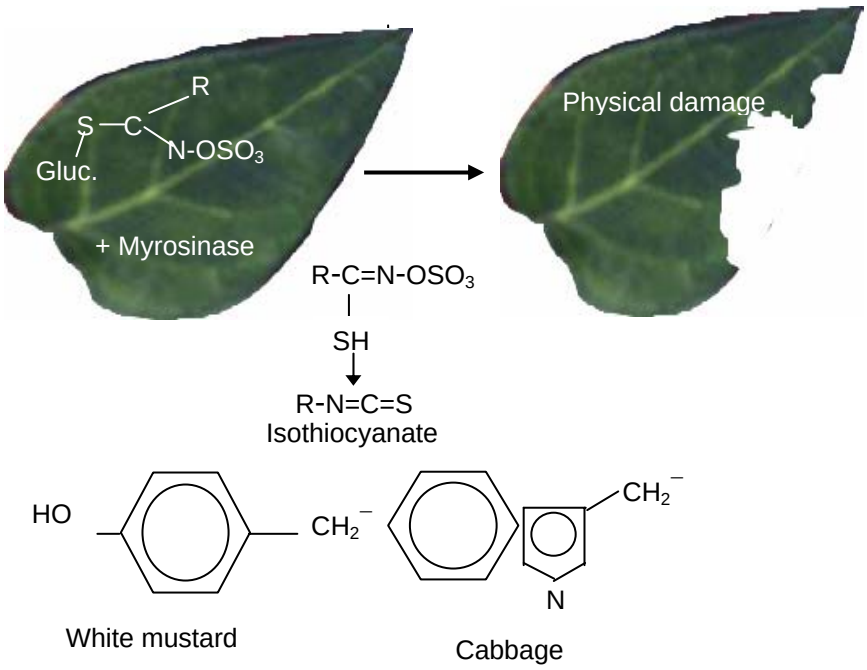

Figure 2.2: Production of plant antimicrobials as a result of physical damage

Some spices are known to contain essential oils that possess antimicrobial activity e.g. eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allspice (pimento) and cloves, allyl isothiocyanate in mustard, eugenol and thymol in sage and carvacrol (isothymol) and thymol in oregano etc. As a consequence, herbs and spices may contribute to the microbiological stability of foods in which they are used. It has, for example,

been claimed that inclusion of cinnamon in raisin bread retards mould spoilage. However, in some cases, they can be a source of microbial contamination leading to spoilage or public health problems. Outbreaks of botulism associated with crushed garlic in oil and home canned peppers demonstrate that even in relatively high concentrations plant antimicrobials are not a complete guarantee of safety.

These antimicrobial components differ in their spectrum of activity and potency. They are present at varying concentrations in the natural product and are frequently at levels too low to have any effect. Humulones contained in the hop resin impart the characteristic bitterness of the product but have also been shown to possess activity against the common beer spoilage organisms, lactic acid bacteria.

Antimicrobial oleuropein from green olives and its aglycone are also inhibitory to lactic acid bacteria and if not removed at this early stage, they would prevent the necessary fermentation occurring subsequently. The hydroxycinnamic acid derivatives *(*coumaric, ferulic, caffeic and chlorogenic acids) found in fruits, vegetables, tea, molasses and other plant sources all show antibacterial and some antifungal activity.

Animal products too, have a range of non-specific antimicrobial constituents, for example egg white or albumen possesses a variety of inhibitory components (Table 2.4). Similar factors can also be found in milk, however, in lower concentrations e.g. enzyme lysozyme which catalyses the hydrolysis of glycosidic linkages in peptidoglycan. Destruction or weakening of this layer causes the cell to rupture (lyse) under osmotic pressure. Lysozyme is most active against gram- positive bacteria, where the peptidoglycan is more readily accessible, but it can also kill gram- negatives if their protective outer membrane is damaged in some way.

Table 2.4: Antimicrobial substances in egg and milk

Egg	Milk
Ovotransferrin (conalbumin)	Lactoferrin
Lysozyme	Lysozyme
Avidin	-
Ovoflavoprotein	-
Ovomucoid and ovoinhibitor	-
-	Lactoperoxidase
-	Immunoglobulin

Other components limit microbial growth by restricting the availability of key nutrients. Ovotransferrin and conalbumin in egg white and lactoferrin in milk are proteins that scavenge iron from the medium. Iron is an essential nutrient for all bacteria. Infact, lysozyme with conalbumin provides fresh eggs with a fairly efficient antimicrobial system. In addition, egg white has powerful cofactor-binding proteins such as avidin and ovoflavoprotein, which remove biotin and riboflavin restricting the growth of those bacteria for which they are essential nutrients.

Cows' milk contains several other antimicrobial substances including conglutinin, lactenins, anticoliform factor and the lactoperoxidase system.

Casein as well as some free fatty acids that occur in milk have also been shown to be antimicrobial.

2.7.2 Inhibitory Substances Developed/ Destroyed in Food due to the Activity of Microorganisms

Microorganism growing in food may produce one or more substances inhibitory to other organisms, products such as acids, alcohols, peroxides, or even antibiotics. Propionic acid produced by the propionibacteria in Swiss cheese is inhibitory to molds; alcohol formed in quantity by wine yeasts inhibits competitors; and nisin a polypeptide produced by *Streptococcus lactis* may be useful in inhibiting lactate fermenting, gas-forming clostridia during curing of cheese. These may however, be undesirable during the manufacturing process since these would slow down some of the essential lactic acid streptococci. *Streptococcus cremoris* produces an inhibitor named diplococcin. The most pathogenic member of genus - *S. pyogenes* forms an inhibitor, streptococcin A-FF22. Streptococcin A-PF22 had many properties in common with nisin.

Gram-negative organisms and molds are insensitive to nisin. However, its effectiveness against sensitive gram-positive organisms depends on the bacterial load. As the number of organisms increases, the inhibitory effectiveness of nisin decreases. Nisin can be used along with heat processing since heat treated spores become more nisin sensitive. Thus, sterility might be attained with less heat treatment than presently used thereby decreasing the fuel consumption.

In addition to inhibitory polypeptides and bacteriocins, lactic streptococci produce acids and peroxides. These add up to a formidable array of substances designed to hinder and suppress other microbes. Hence, lactic acid bacteria are excellent competitors in foods. *S. diacetylactis* produces inhibitor inhibiting a broad-spectrum of gram-positive and gram-negative organisms. The undissociated molecule is the toxic component.

There is also the possibility of the destruction of inhibitory compounds in foods by microorganisms. Certain molds and bacteria are able to destroy some of the phenolic compounds that are added to meat or fish by smoking or benzoic acid added to foods; yeasts resistant to it destroy sulfur dioxide; and lactobacilli can inactivate nisin.

2.7.3 Inhibitory Substances Developed During Processing of Food

Heating foods may result in the formation of inhibitory substances e.g. heating lipids may hasten autoxidation and make them inhibitory and browning concentrated sugar syrups may result in production of furfural and hydroxymethylfurfural, which are inhibitory to fermenting organisms. Milk also has the capacity to generate antimicrobials in the presence of hydrogen peroxide. The milk enzyme lactoperoxidase will catalyse the oxidation of thiocyanate by hydrogen peroxide to produce *inter alia* hypothiocyanate. This can kill gram-negative bacteria and inhibit gram-positives, possibly by damaging the bacterial cytoplasmic membrane.

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. Enlist the various mechanisms of action of inhibitory substances.

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2. Elaborate on the inhibitory substances naturally present in plants with suitable examples.

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3. Enlist all the antimicrobial constituents present in egg and milk.

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4. What is the mode of action of lysozyme?

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5. Give examples of inhibitory substances developed in food due to the activity of microorganisms.

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2.8 LET US SUM UP

Food is the substrate for growth of microorganisms. Since our foods are of plant and animal origin, all the characters of plant and animal tissues that affect the growth of microorganisms are important. In other words, food will dictate what grows or does not grow on it. Thus, knowledge about the factors that favour or inhibit growth of microorganisms is a must to make predictions about the microflora that may develop. The main factors, which influence microbial activity are pH, water activity, redox potential, nutrient composition, biological structure and presence of inhibitory substances in foods.

Each microorganism has a minimal, maximal and optimal range for pH, water activity and redox potential at which they can grow. These in turn would determine the microflora of food. Buffers in food and poising capacity of food also play an important role in deciding the succession of microorganisms and the extent of spoilage occurring in food.

Different microorganisms have different nutritional requirements. Some have a wide range while others are very fastidious in their nutritional requirements. The inability of an organism to utilize a component of food limits its growth and others with not so stringent requirements gain a competitive edge over it and predominate, thereby typifying the natural microflora of that food.

Plants and animals have also evolved mechanism of defense against invasion and proliferation of microorganisms. Antimicrobial barriers allow food to remain relatively free from microorganisms and the other inhibitory substances present in food/ produced during invasion by microorganisms tend to maintain low microbial counts in food.

These six parameters represent nature's way of preserving plant and animal tissue from microorganisms. All these parameters have an important role to play in determining microbial ecology of food. These in turn decide the type of microbial activities likely to occur and the type of spoilage occurring. All these factors are interlinked and the changes in one factor may affect microbial requirements. By taking these natural phenomena into account, one can make effective use of each or all in preventing or retarding microbial spoilage of the products that are derived from them.

2.9 KEY WORDS

Aerobic **:** microorganisms are those, which require free oxygen for growth.

Anaerobic : microorganisms are those, which grow best in the absence of free oxygen.

Antibiotics : are substances produced by microorganisms which inhibit the growth of other microorganisms.

Antimicrobials : are substances which inhibit the growth of microorganisms.

Bacteriocins : are substances produced by a strain of bacterial spp. which inhibit growth of other strains of that bacterial spp.

Buffers : are the compounds that resist changes in pH.

Facultative : microorganisms are those, which grow well either aerobically or anaerobically.

Halotolerant : are those microorganisms, which are able to grow in the presence of high concentrations of salt.

Osmotolerant : are those microorganisms, which are able to grow in the presence of high concentrations of unionized organic compounds such as sugars.

Oxidising agent : is a substance that readily takes up electrons.

Phytoalexins : are a class of antimicrobials which are produced by many plants in response to microbial invasion**.**

Poising capacity : is the ability of food to resist change in a food's redox potential.

Proteolytic microorganisms : are those, which are able to hydrolyze proteins.

Redox potential : of a substrate may be defined generally as the ease with which the substrate loses or gains electrons.

Reducing agent : is a substance that readily gives up electrons.

Standard redox potential, *E*o' : is the tendency of an atom or molecule to accept or donate electrons.

Water requirement or water activity a_w : is the vapour pressure of the solution (of solutes in water in most foods) divided by the vapour pressure of the solvent (usually water).

Xerotolerant : are those microorganisms which are able to grow on dry foods.

2.10 ANSWERS TO CHECK YOUR PROGRESS EXCERCISES

Check Your Progress Exercise 1

1. Fish is spoilt more rapidly than meat under chilled conditions. The pH of post-rigor mammalian muscle, around 5.6, is lower than that of fish (6.2-6.5) and this contributes to the longer storage life of meat. Those fishes that have a naturally low pH such as halibut (pH~5.6) have better keeping qualities than other fishes.

2. Your answer should include the following points:

   - pH attained upon rigor mortis of well rested animals
   - not much change in pH change in case of fatigued animal

3. Biological acidity is the acidity developed in food due to the activity of microorganisms growing in it. This generally occurs due to accumulation of lactic acid during fermentation.

4. Your answer should include the following points:

   - partial dissociation of weak acids
   - free passage of undissociated lipophilic acids into the cytoplasm
   - dissociation of lipophilic acids in the microbial cell
   - maintenance of internal pH by expulsion of protons

5. When microorganisms are grown on either side of their optimum pH range, an increased lag phase results. Adverse pH affects at least two aspects of a respiring microbial cell: the functioning of its enzymes and the transport of nutrients into the cell.

Check Your Progress Exercise 2

1. Your answer should include the Halotolerant, Xerotolerant and Osmotolerant microorganisms.

2. Water activity a_w, is the vapour pressure of the solution (of solutes in water in most foods) divided by the vapour pressure of the solvent (usually water).

3. Your answer should include the following points:

   - Solutes and ions tie up water in solution
   - Hydrophilic colloids (gels) make water unavailable
   - Water of crystallization or hydration is usually unavailable to microorganisms

4. Your answer should include the following points:

   - Kind of solute employed to reduce the a_w
   - Nutritive value of the culture medium
   - Temperature
   - Oxygen supply
   - pH
   - Inhibitors

Check Your Progress Exercise 3

1. The tendency of a substrate to accept or donate electrons, to oxidize or reduce, is termed its redox potential (*E*h).
2. Your answer should include aerobic, anaerobic and facultative microorganisms.
3. Poising capacity is the resistance to change in a food's redox potential with change in the redox conditions.
4. Your answer should include the following points:
    - Heating destroys/ alters reducing and oxidizing substances
    - Processing removes reducing and oxidizing substances
5. Factors affecting redox potential of foods are:
    - Redox couples present
    - Ratio of oxidant to reductant
    - pH
    - Poising capacity
    - Availability of oxygen
    - Microbial activity

Check Your Progress Exercise 4

1. Your answer should include the following points:
    - Concentration of sugars in food and their osmotic effect
    - Molds grow at high concentration of sugars
    - Osmophilic bacteria and yeasts grow at high concentration of sugars

Check Your Progress Exercise 5

1. Your answer should include the following points:
    - Type of physical barriers about food
    - These barriers provide inhospitable environment for the microbe
    - They determine type and rate of spoilage
2. Your answer should include the following points:
    - Physical damage allows microbial invasion into tissues
    - It distributes spoilage microbes
    - It releases juices from plant and animal tissues for microbial growth

Check Your Progress Exercise 6

1. The mechanism of action for nearly all antimicrobials can be classified into one or more of the following groups:
    a) Reaction with the cell membrane,
    b) Inactivation of essential enzymes, or

c) Destruction or functional inactivation of genetic material.

2. Your answer should include the following points:

   - Isothiocyanates – mustard oil
   - Thiosulfinates – in garlic, onions and leeks e.g. allium

3. Your answer should include the following points:

   - Antimicrobial substances present in egg
   - Antimicrobial substances present in milk

4. Your answer should include the following points:

   - Lysozyme catalyses the hydrolysis of glycosidic linkages in peptidoglycan
   - Destruction leads to cell lysis
   - Very active against gram positive bacteria

5. Your answer should include the following points:

   - Various types of inhibitory substances produced by microbes
   - Inhibitory substances produced by propionibacteria
   - Inhibitory substances produced by lactic streptococci

2.11 SOME USEFUL BOOKS

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UNIT 3 INDUSTRIALLY IMPORTANT YEAST, MOLD AND BACTERIA

Structure

3.0 OBJECTIVES

After reading this unit you should be able to:

- state the fermenter, types of microorganisms involved in fermentation and their processes;
- explains the different products made by fermentation; and
- describe the waste from food processing industry and their utilization.

3.1 INTRODUCTION

Microorganism on one hand are responsible for causing a number of diseases, on the other hand they are employed to produce a number of useful products. These useful microorganisms include an array of yeasts, molds and bacteria. Traditionally, the man prepared wine, curd, vinegar and pickles using fermentation. Earlier, the term 'Fermentation' was used for the production of wine but at present it encompasses the foods made by the application of microorganisms including lactic acid bacteria (LAB). Lactic acid fermentation is one of the oldest method of preserving fruits and vegetables. Apart from contributing certain desirable physical and flavour characteristics, it also prolongs the availability and processing period of the products at relatively low cost. Many of the fermented products are made at industrial scale making use of microorganisms. Some of the fermented products and industrially important microorganisms are listed in Table 3.1.

Table 3.1: Production/synthesis of various compounds by micro-organisms

Product	Micro-organism(s) involved
Alcoholic beverages & related products	
Beer	*Saccharomyces cerevisiae*; *S. carlsbergensis*
Bourbon whiskey	*S. cerevisiae*
Cider	*S. cidri*
Palm wine	*Acetobacter s*pp.; yeasts
Sake	*Aspergillus oryzae*; *Lactobacillus* spp.; *Leuconostoc* spp.; *S. cerevisiae*
Scotch whiskey	*S. cerevisiae*
Thumba	*Endomycopsis fibuliges*
Tibi	*Betabacterium vermiforme; S. intermidium*.
Vinegar	*Acetobacter* spp.
Wines	*S. cerevisiae* var. *ellispoideus*
Breads	
Idli	*Leuconostoc mesenteroids*
Rolls, cakes etc.	*S. cerevisiae*
Colour	
β-carotene	*Blakeslea trispora; Rhodotorula spp.*
Astaxanthine	*Phiffia rhodozyma*
Dairy products	
Acidophillus milk	*Lactobacillus acidophilus*
Bulgarian milk	*L. bulgaricus*
Cheeses (brie,	*Streptococcus lactis; S. cremoris*: *camembert, cheddar, S. durans; Penicillium camembertii*: *edam, P. candidum; P. roquefortii; Lactobacillus caseiroqueforte)*
Kefir	*Streptococcus lactis; L. bulgaricus; torula* spp.
Kumiss	*L. bulgaricus; L. leicuhmannii; Torula* spp.
Yoghurt	*L. bulgaricus*
Enzymes	
Amylases	*Bacillus* spp.; *Aspergillus niger; A. oryzae.*
Cellulases	*Trichoderma reesei.*
Glucose oxidases & catalase	*Corynebacterium* spp.
Invertase	*S. cerevisiae*
Lipase	*Saccharomycopsis lipolytica.*
Pectinases	*Aspergillus* spp.
Proteases	*B. licheniformis*; *B. subtilis; Aspergillus* spp.; *S. cerevisiae*

Meat and fishery products

Country cured hams	*Aspergillus; Penicillium* spp.
Dry sausages	*Pediococcus cerevisiae*

Microbial cells as fermented products

Bakers' yeast	*Saccharomyces cerevisiae*
Single cell	*Candida utilis; C. arborea;* protein (SCP) *Methylophilus methylotrophus; Saccharomycopsis lipolytica; Spirulina*
Mushrooms	*Agaricus bisporus*; *Morchella hortensis*

Non-beverage plant products

Miso	*Aspergillus oryzae*
Saurkraut	*Neurospora sitophila*
Sufu	*L. delbrueckii*
Tempeh	*A. oryzae; Rhizopus oligosporus; R. oryzae*

Organic acids

Acetic acid	*Acetobacter aceti; C. aceticum*
Citric acid	*Aspergillus niger; Saccharomycopsis lipolytica*
Lactic Acid	*Lactobacillus delbrueckii*

Polysaccharides

Alginate	*Azotobacter vinelandii; Pseudomonas aeruginosa*
Dextrans	*Leuconostoc mesenteroids; Klebsiella; Acetobacter*
Pullulan	*Aureobasidium; Pullularia* spp.

Vitamins and Amino acids

Riboflavin	*Eremothecium ashbyi*
Vit. B-12	*Bacillus megaterium; Streptomyces olivaceus, Propionibacterium*
Pro-Vit. A.	*Rhodotorula gravillis.*

3.2 CULTURING OF IMPORTANT MICROORGANISM

The human food supply consists basically of plants and animals or products derived from them, so our food supply can contain microorganisms in interaction with the food. The interactions between microorganisms and our food is beneficial as exemplified by many cultured products developed by fermentation and are consumed and enjoyed by many people (Bread, bear, wine etc). To produce such products microorganisms are added as pure culture or mixed cultures. However, in some cases no cultures may be added if the desired microorganisms are known to be present in sufficient numbers in the original raw material.

Starter culture, pure as well as mixed are usually employed in the manufacture of certain fermented food and dairy products. Cultures for food fermentations are selected primarily on the basis of their stability and their ability to produce desired products. Mother culture is usually prepared daily from a previous

mother culture and originally from the stock culture. The mother cultures can be used to inoculate a large quality of culture medium to produce the mass or bulk culture to be used in the fermentation process.

Bacterial cultures: Most of the bacterial cultures employed as starters are for dairy products. Sausage and bread also use pure or mixed cultures of lactic acid bacteria (LAB) e.g. *Streptococcus lactis* sub sp *lactis, S. mesenteroides* sub-sp. *cremoris* etc.

Yeast cultures: Most yeasts of industrial importance are of the genus *Saccharomyces*. It is used to manufacture wine, beer and other alcoholic products.

Bakers' yeast: Yeast for baker's yeast production: Strains of *S.cerevisiae, S.uvarum* are used.

Wine yeast: *S. cerevisiae var. ellipsoidus* (Plate 1.1)

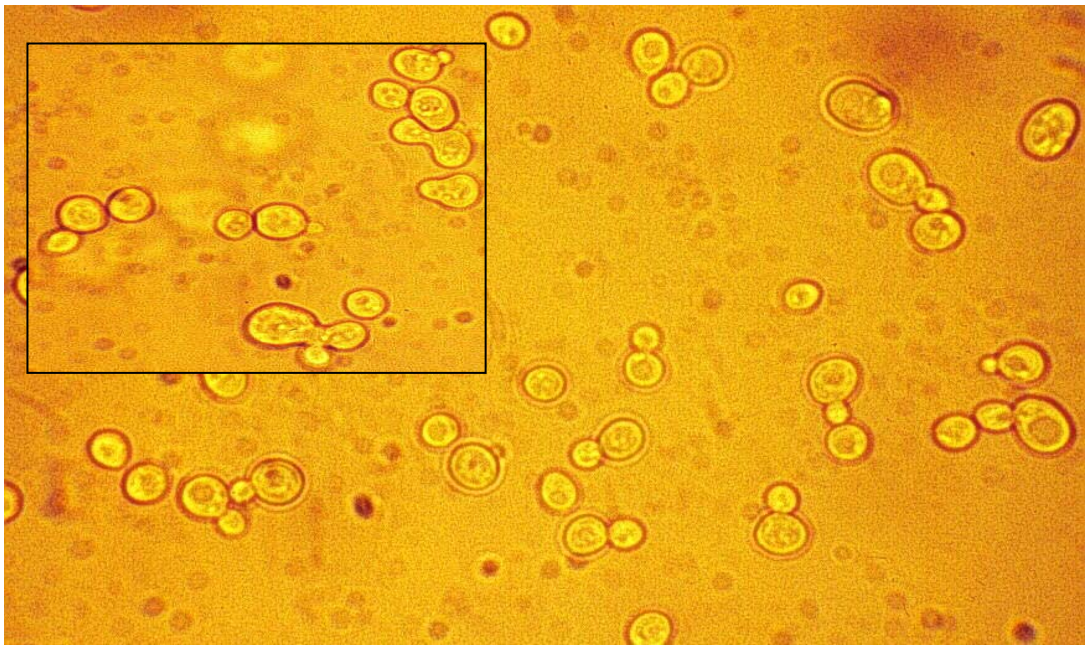

Plate 1.1: Photo micrograph of yeast cells showing budding (inset yeast cells are mating)

Distillers yeast: High alcohol yielding strains of *S.cerevisiae* var *ellipsoidus*.

Mold cultures: Stock of cultures of molds usually are carried in slants of a suitable agar medium and may be preserved as spore stab for a long period by freeze drying (*Penicillium roquefortii).*

3.3 ENZYMES AND KINETICS

Enzymes: Enzymes are biological catalysts possessing extraordinary efficiency, specificity and are mostly protein in nature. Enzyme commission has classified various enzymes on the basis of the type of the reactions catalysed. All the enzymes have been classified into 6 classes.

Class 1 : Oxido-reductase
Class 2 : Transferase
Class 3 : Hydrolase
Class 4 : Lyase
Class 5 : Isomerase
Class 6 : Ligase

Properties of Enzymes

- All enzymes are protein in nature except nucleases.
- All enzymes are specific in their functions.
- Enzymes are sensitive to temperature i.e. they are functional at optimal temperature.
- These are destroyed at higher temperature.
- Enzymes are not destroyed during their use.

Kinetics of Enzyme Reactions

The studies on the kinetics of enzyme reactions must be based on quantitative measurements of the rate of the catalyzed reactions. Main factors which influence the kinetics of enzymatic reactions are as follow:

Enzyme concentration: The velocity of enzymatic reactions is directly proportional to the concentration of the enzyme proteins.

Substrate concentration: When the velocity of the reaction is plotted vs. substrate concentration, classical enzymes give a rectangular curve.

pH: Almost every enzyme exhibits maximum activity at a particular pH which is called optimum pH.

Temperature: Almost every enzyme exhibits an optional temperature at which the enzyme exhibits maximal activity. A graph of enzyme velocity versus temperature, is a bell shaped curve.

Role of enzymes in food processing: The enzymes play a significant role in food processing. Pectinase enzymes are used in juice clarification (apple juice, guava juice), in softening of fruit (apple, tomatoes, peaches, avocadoes), and thereby resulting in increase in yield of juices and pulps extraction of juice from fruits. Proteases (papain) results in clarification and removal of cloudiness in beer and wine. Glucose oxidase enzyme is used in removal of glucose from egg white and thereby, improve, the colour of dehydrated egg powder. Pectinase with cellulase has been employed for extraction of oil from oil containing fruits (olive). Enzyme diastase converts starch to sugar during beer preparation.

3.4 TYPES OF FERMENTATION

Fermentations have been classified on the basis of relationship of the formation of product, substrate utilization or the free amount of water as given below:

Solid state fermentation: Fermentation processes which take place in the absence or near absence of free water in the substrate are termed as solid state fermentation (SSF). It is imperative, however, that the substrate contain enough moisture absorbed in the substrate particles within the substrate. SSF have been used mostly for food fermentation and production of a few enzymes.

Extractive fermentation: There are several industrially important products being catalysed by enzymes which are susceptible to end product or feedback inhibition. Hence, the increased concentration of the product inhibits the enzymes involved in its own synthesis so that the overall rate of conversion of substrate to the desired product is lowered. When the end product or anyone of

the by-product of fermentation interacts with the enzyme, the synthesis of the final product proceeds sub-optimally and in extreme case may stop altogether. This problem has largely been overcome by using a technique called extractive fermentation. In it there is fast removal of product, or by-product of a metabolic pathway, so that their subsequent interference with the cellular or medium component is not possible. Hence, it involves all the actions taken for the separation of a product from its producing cell. Separation of the product can be achieved either inside the reactor (internal) or outside the reactor (external).

Submerged fermentation: Fermentation processes which take place in the presence of free water in the substrate are termed as sub-merged fermentation. Such fermentations have been used mostly to the produce fermented food and beverages.

3.5 TYPE OF FERMENTER, CONCEPT OF BATCH AND CONTINUOUS FERMENTATION

Fermenter: The industrial usage of micro-organisms often requires that they be grown in large vessels containing considerable quantities of nutritive media. These vessels are commonly called fermenters. Therefore, fermenter is the basic equipment of fermentation.

Types of fermenters: Some of the types of fermenter are listed below:

1. Shake flasks and bottles
2. Stirred tanks
3. Air-lift fermenters
4. Tower fermenter
5. Rotating disc fermenter
6. Fixed bed fermenter
7. Fluidized bed fermenter

Batch fermentation: In this fermentation, starter culture is added to the medium and the product is withdrawn only after completion of fermentation.

Continuous fermentation: In this fermentation, the substrate is continuously fed to the fermenter and the product is also withdrawn continuously.

3.6 MICROBIAL PRODUCTION OF WINE, VINEGAR, SAUERKRAUT, ETHYL ALCOHOL, BEER, ORGANIC ACID

3.6.1 Wines

The term ‘wine’ is applied to a beverage made by alcoholic fermentation of grape or grape juice and final production is obtained without distillation. But now-a-days, any fleshy fruit or flower in the new world may be employed for this purpose. Wine was suggested to have been made during the Neolithic period in the near East. These are the part of food of man ever since his settlement in Tigoris Euphorates basins and have also been used as a therapeutic agent.

Wines are produced by the fermentation of juices/extracts of many fruits such as apple, pear, cherries, most of berries, rhubarb, dandelion, honey, besides bananas, pineapple, cashew nut, pomegranate, lemons, tangerines, oranges, dates and figs. Wines from grapes are classified basically into red and white wines.

Types of Wines

Still wines: These wines retain none of the carbon dioxide produced during the fermentation.

Sparkling wines: These are the wines which have considerable amount of carbon dioxide. Champagne in France is the sparkling wine made in Champagne region.

Dry wines: These wines contain little or no unfermented sugar.

Sweet wines: Wines having either unfermented sugar or with added sugar later on are called sweet wines. Both types of wines generally contain 11 to 14% of alcohol.

Fortified wines: Wines to which distillate of wine called "Brandy" is added and may contain 15 to 21% of alcohol.

Table wines: It is a wine having comparatively low alcohol content (7 to 11%) and little or no sugar.

Sherry: It is produced by special processing technique from wine, containing 18 to 21% alcohol and could be sweet or dry.

Cider: Cider is a low alcoholic beverage obtained from apple by fermentation.

Perry: It is a wine made from pear juice.

Mead: This type of wine was prepared by the Indians from honey.

Vermouth: Wine flavoured with a characteristic mixture of herbs and spices, some of which impart an aromatic flavour and odour while others a bitter flavour. It can be sweet or dry with alcohol content of 15 to 21%.

Toddy: Sweet alcoholic drink, having alcohol content of 4-6%, is made by the fermentation of sap from coconut palm.

Pulque: National drink of Mexico, contains 6-7% alcohol and B-vitamins.

Method of Table Wine Preparation

Grape is the most widely used fruit to make wine but it can be prepared from any fruit having fermentable sugars, optimum acidity, nitrogenous compounds or other growth factors to make wine of acceptable quality. The major difference is in the extraction of sugar from the pulp of some fruits. From grape, red and white wines are produced the world over using black/red coloured and white varieties, respectively. The generalized flow sheets for wine making from grapes is shown in Figure 3.1.

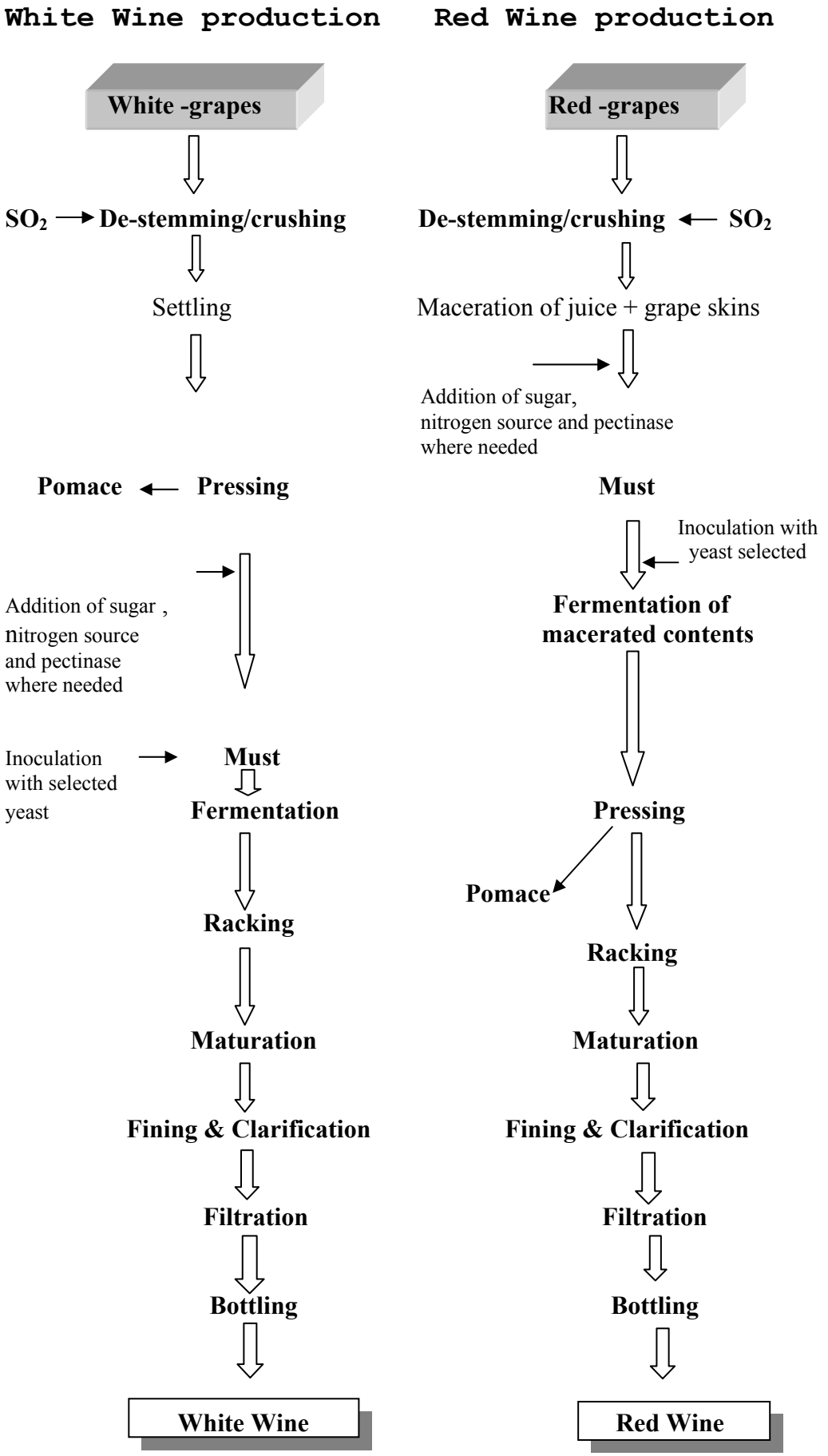

Figure 3.1: Flow–sheet of process to make red and white wine

Preparation of must: For wine preparation, the first step is the preparation of must which is prepared depending upon the type of fruits used and the type of wine to be made. Must is a juice or pulp corrected for sugar, acid/pH, nitrogen source or other requirements for the alcoholic fermentation. To prepare the must, the fruits are trimmed and washed and the must Juice is extracted or fruit is made into pulp. In the preparation of white wine only the free run juice is used while in the red wine, the skin and seeds along with pulp/juice are fermented together for some time to get attractive coloured wine. Proper dilution of fruit pulp is required as fruits like plum and apricot are highly acidic and effect the fermentability besides making the wine unpalatable. The sugar content of the juice or pulp is checked with an instrument called refractometer and is expressed as degree Brix. Sulphur dioxide ($S0_2$) is added to the must to control the wild microflora and to allow the yeast to act efficiently to conduct the alcoholic fermentation. Amelioration (or correction) of must for better fermentability with ammonium salt and vitamins like thiamine, biotin is necessary in some fruits.

Preparation of active yeast culture: An active culture of wine yeast (*Saccharomyces cerevisiae* var *ellipsoideus*) is prepared from the stock culture in the juice to be used for wine making.

Fermentation: After must preparation, activated yeast starter culture is added to the must and fermentation is carried out at a temperature of 20-25^{o}C, till the sugar content or the ^{o}Brix stabilizes.

Siphoning/racking

Siphoning or racking is a simple but important process wherein the wine is transferred through a clean pipe into another container, kept at a lower height than the vessel with wine. It is done after completion of fermentation. Two or three rackings are usually done at an interval of 15-20 days to separate the yeast and other settled materials at the bottoms of container.

Maturation

As the newly made wine is harsh and has yeasty flavour maturation (from 6 months upto a year) is allowed to make the wine mellow (It is the term used to signify the sensory quality of wine having smoothness i.e. is devoid of any harsh taste)in taste and fruity in flavour.

Clarification: Clarification of wine is done by using filter aids such as bentonite, celite and tannin/gelatin using a machine called filter press.

Blending: Blending is also practiced in some cases to make wine sweet or better flavoured before pasteurization.

Pasteurization: Wine is generally pasteurized at a temperature of 62^{0}C for 15-20 min, after bottling.

Storage: Low temperature storage is preferred for good quality wine.

3.6.2 Beer

Beer is an alcoholic beverage primarily prepared from barley besides other cereals in limited quantities and is consumed in large quantities throughout the world. Beer and ale the principal malt beverages made with hops, yeast, water and malt adjuncts. Adjuncts are the malted cereals other than barley, used in

minor quantities. Brewing was one of the earliest processes undertaken on a commercial scale and became one of the first process that has developed from an art into a technology. Beer can be differentiated from ale as in beer bottom fermenting yeast is employed while in ale the top fermenting yeast is employed. In the preparation of ale, more hops is used. It is usually pale yellow in colour, tart in taste and have more alcohol content. On the basis of alcohol content beers can be classified as light beer having 3-5% v/v and hard beer having 5-8% alcohol content. Beer production is divided into four distinct process as described here.

Malting: It is obtained by soaking followed by germination of barley or other cereals and drying of the germinated cereal. Then, most of sprouts or germs are removed and the malt remains. The malt is crushed before its use in beer making.

Mashing: It is the process in which extraction of the ground malted barley with water is made. The mashing is done so as to make soluble as much as possible of the valuable constituents of the malt and malt adjuncts. It causes hydrolysis of starches, other polysaccharides and proteins. The insoluble material is then filtered. The liquid so obtained is called wort.

Wort boiling: Boiling of wort with hops (Hops is the female flowers of hops plant used in beer production to give flavour and bitter taste) is carried out to concentate the wort, inactivate the enzymes, extract soluble substances from the hops,coagulate and precipitate the proteins and other substances, caramelize sugar slightly and to contribute antiseptic substances (Chiefly the alpha resins humulone, co-humulone and adhumulone) to the wort and beer.

Fermentation: A special beer, bottom fermenting yeast strain *Saccharomyces cerevisiae* var *carlbergensis*, is used for the inoculation or pitching of the cooled wort. The wort temperature during the fermentation varies in different breweries but is usually in the range from 3.3 to 14^{o}C. The fermentation is usually completed within 8 to 14 days. During fermentation as the carbon dioxide is evolved in increasing amounts, the foaming increases; later it decreases to none when the fermentation is finished. At the later stage, the bottom yeast flocculates and settles down.

Aging or Maturation: The young, green or draft beer is stored or lagered in vats at about O^{o}C for several weeks to several months, during which period precipitation of proteins, settling of yeast, resin and other undesirable substances takes place and the beer becomes clear and mellowed or matured.

Finishing: After aging, the lager beer is carbonated to a CO_2 content of about 0.45 to 0.52 per cent, mostly by means of gas collected during the fermentation or by addition of CO_2 from cylinders. Then, beer is cooled, clarified or filtered and packaged in the bottles, cans or barrels.

3.6.3 Vinegar

The word vinegar is derived from two French words, *vin* and *aigre* meaning sour wine but the term is used to denote a condiment prepared from various sugar and starch containing materials by alcoholic and subsequent, acetic acid fermentation. It is one of the several fermented foods prepared and consumed by early man, even today. Earlier, it was used as a beverage, a condiment, a preservative, a household cleansing and medicinal agent. Vinegar mainly consists of a dilute solution of acetic acid in water, also contains colour,

flavour and extracted substances besides fruit acids, esters and inorganic salts which vary according to its origin. The minimum legal strength for vinegar is 4% acetic acid (w/v).

Types and Composition of Vinegar

1. **Synthetic vinegar:** This type of vinegar is directly prepared from synthetic acetic acid with the addition of water and finally, it is coloured by caramel.

2. **Brewed vinegar:** Virtually, anything having enough sugar to produce alcohol can be used to make brewed vinegar. The vinegar usually derives its descriptive name from the material from which it is made such as: cider vinegar is made from apple juice, alegar from ale, malt vinegar from malted grains spirit vinegar from alcohol etc.

Vinegar Preparation

It involves two step fermentations as detailed below:

Alcoholic fermentation: The first is alcoholic fermentation, mainly carried out by yeast *Saccharomyces cerevisiae* either by pure culture inoculation or by the natural process of fermentation. The process can be represented by a simplified equation:

$$C_6H_{12}O_6 \rightarrow 2CO_2 + 2C_2H_5 - OH + 55 \text{ Kcal}$$

Glucose Carbon dioxide Ethyl alcohol

In the process, ethyl alcohol is not the only product but small amounts of other compounds like glycerol, succinic acid, amyl alcohol, propyl alcohol etc. are also produced in this fermentation. The fermentation is anaerobic.

Acetous fermentation: The second fermentation is acetic acid fermentation. It is an oxidative fermentation carried out by acetic acid bacteria like *Acetobacter aceti*. In the vinegar production, pure culture of acetic acid bacteria is not used, due to more efficiency of mixed cultures. The oxidation reaction can be shown as:

$$C_2H_5OH + O_2 \rightarrow CH_3COOH + H_2O + 116 \text{ Kcal}$$

Ethyl alcohol Acetic acid

The optimum temperature of fermentation is 26^oC which is achieved by the heat generated in the process.

Process of Vinegar Preparation

Slow process: This process takes a long period and is generally followed in countries like India. The juice kept in the barrels is allowed to undergo alcoholic and acetic fermentations slowly with the passage of time. The bung hole of the barrel is covered with a piece of cloth to screen-off the dust and flies, and the barrel is placed in a damp but warm place. It takes about 5-6 months to complete the whole alcoholic and acetous fermentation to produce the vinegar from the juice. The main drawbacks of this process are: alcoholic fermentation is often incomplete, the acetic fermentation is very slow and the yield is low coupled with an inferior quality vinegar.

Quick process: In the quick process like generator process alcoholic liquid is in motion and this process is applied mostly to the production of vinegar from spirit (alcohol). Fruit or malt liquors are well supplemented with food for the vinegar bacteria, but to maintain active vinegar bacteria in generator methods using alcohol denatured with ethyl acetate or vinegar, it must be supplemented with a combination of organic and inorganic compounds known as vinegar food. Combinations of substances such as dibasic ammonium phosphate, urea, peptones, yeast extract, glucose, malt, starch, dextrin, salts etc have been made. Materials such as pumice, branches of vines and grape stems for packing the generators are used. Schiizenbach introduced the use of a vat instead of cask for the acetification process and provided mechanical means for the repeated distribution of the acidic liquid over the packing.

Generator: The equipment used is known as "Upright Generator" which in its simplest form is a cylindrical tank that comes in different sizes and is usually made of wood. Its interior is divided into 3 parts:

i) **Upper section:** Here, alcoholic liquid is introduced.

ii) **Large middle section:** In this section, liquid is allowed to trickle down over beech wood shavings, corn cobs, charcoal, coke, or some other material that will provide a large total surface area yet not settled into a compact mass.

iii) **Bottom section:** This section is for the vinegar collection.

The alcoholic liquid is put at the top through an automatic feed trough or a sprinkling device (sparger) and trickled down over the shavings or other material on which a slimy growth of acetic acid bacteria has been developed and the bacteria oxidize the alcohol to acetic acid and the process is called acetification. Air enters through the false bottom of the middle section and after becoming warm, it is exhausted out through a ventilation above. As considerable heat is released by oxidation process, it is necessary to control the temperature below 30^{o}C. It is usually done by using cooling coils, by adjusting the rate of alcoholic liquid, feeding air and by cooling the alcoholic liquid before it enters the generator or by cooling the partially acetified liquid that is returned to the top from the bottom section of the tank for further acetification.

3.6.4 Lactic Acid Bacteria (LAB) and Fermented Foods

Lactic acid bacteria (LAB) are obligate microorganisms producing lactate from sugars as the main end product, besides producing inhibitory substances like organic acids, bacteriocin, hydrogen peroxide which are antagonistic towards other microorganisms. Fermented dairy products are known to be inhibitory to both pathogenic and spoilage causing microorganisms and Yoghurt is the best known fermented milk product (with fruit pulp).Cultured milk and milk products contain lactic acid bacteria that prevent the occurrence of stomach, colon and other cancers.

Traditional fermentations of vegetables were depended upon growth of naturally occurring lactic acid bacteria to metabolise sugars in the vegetables to mainly lactic acid and improve their taste and keeping quality. However, starter cultures are being used now to develop controlled fermentation

It is established that more than one species of lactic acid bacteria are responsible for vegetable fermentation. Lactic acid bacteria responsible for natural fermentation of vegetables are within the genera of *Streptococcus*, *Leuconostoc, Pediococcus* and *Lactobacillus*. Acidity, pH, salt concentration, temperature, naturally occurring inhibitors, chemical additives, exposed brined surface to air and sunlight, amount of fermentable carbohydrates in the vegetables and availability of nutrients in the brine are important factors affecting the lactic fermentation.

Sauerkraut

It is the clean, sound product of characteristic flavour, obtained by full fermentation, chiefly lactic of properly prepared and shredded cabbage in the presence of not less than 2% nor more than 3% of salt. It contains, upon completion of the fermentation not less than 1.5 per cent of acid expressed as lactic acid. To prepare sauerkraut rough outer leaves of fully mature solid cabbage heads are removed. Head are quartered, the cores are removed and then, shredded the quarters into thin strips which are mixed with salt. About 2.25 to 2.5% of salt by weight should be added to the shredded cabbage to obtain kraut of the best quality. Pack the cabbage loosely in a jar, place a wooden board on the top. In order to press out juice from the cabbage, a heavy stone is placed on the wooden board. The jar is kept at a warm place (24 to 31^{o}C) for 8 to 12 days to allow fermentation to complete. The brine is separated from the cabbage, boiled and poured hot over the cabbage shreds in the jars. Sauerkraut can be packed in cans also. The cans are filled with the hot juice, exhausted and processed till the temperature at the centre of can reaches 82^{o}C.

Prominent bacteria that attain appreciable number early in fermentation are *Enterobacter cloacea* and *Erwinia herbicola* and contribute some flavour. However, *Leuconostoc mesenteroides* bacteria begins to outgrow all organisms and continue acid production upto 0.7 to 1% (as lactic acid). Next, *Lactobacillus plantarum*, a non-gas forming lactobacilli continues the production of acid and can raise the acidity to 1.5 to 2.0%. These bacteria produce chiefly lactic acid in their fermentation of sugars. A final acidity of 1.7% as lactic acid is most desirable and fermentation can be stopped at this stage by canning or refrigerating the sauerkraut.

Kanji

Carrots of deep purple variety are fermented in Northern India and Pakistan to make a ready-to-serve beverage /drink called as *Kanji.* It is a popular beverage and is considered to have cooling and smoothing properties besides nutritional content. To prepare it, the carrots are washed, grated finally. For every Kg of grated carrot, 7Kg of water, 200g of salt, 40 g of crushed mustard seeds and 8g of hot chillies are added followed by placing the mixture in a glazed earthenware, leaving a tiny whole for the release of gases produced during fermentation. The mixture is fermented for 7-10 days. It is strained through a muslin cloth. The final product is acidic in taste with an attractive purple red colour and is usually consumed within 3-4 days.

Pickles from Vegetables

Vegetables like cucumber are pickled whole or in slices after washing in potable water. For every one Kg of cucumber, 15g salt is added which results in the formation of brine. It is followed by lactic acid fermentation. Depending upon the ambient temperature it takes one to four weeks. The fermented cucumbers are stored in clean capped jars after pasteurization.

Radish can also be pickled in a manner similar to sauerkraut as discussed earlier.

Kimchi

It is a fermented food of Korea with cabbage or radish as the main ingredient. Cucumbers can also be added. Cabbages are cut and brined in 5 to 7% salt solution for 12 hr or in 15% brine for 3 to 7 hr. Then, brined cabbage is rinsed and mixed with 10% seasoning ingredients i.e. garlic, green onions, peppers, ginger, mustard, parseley, sesame grains and fermented shrimp. This mixture is allowed to ferment in jars which takes a few days at temperature of more than 20^{o}C for a month below 10^{o}C. 'Kimchi' has a pH value of 4-4.5 and lactic acid content of 0.4 to 0.8%. The main organisms responsible for fermentation of 'kimchi' are *Leuconostoc mesenteroides* and acidifying microorganism is *Lactobacillus plantarum*.

3.6.5 Ethanol Production

The material rich in sugar can be converted into ethanol. The fermentation is carried out using yeast like *Saccharomyces cerevisiae*. The sugars like glucose is converted into ethyl alcohol and carbon dioxide, anaerobically. Ethanol is a liquid fuel or liquid fuel supplement and is used as a solvent in many industries.

The waste from fruits and vegetable processing industries being rich in polysaccharides (cellulose, hemicellulose and lignin) has been subjected to SSF for the production of ethanol. The cellulose and hemicellulose present in the processing waste like apple pomace are readily fermented by anaerobic bacteria. For ethanol production, the waste from processing industries has to be pre-treated due to presence of lignin. A SSF process has been used for production of ethanol from apple pomace by using *Saccharomyces cerevisiae*. Apple, pear, orange peel and cherry wastes have also been utilized for production of ethanol by fermentation with *Saccharomyces cerevisiae*.

3.6.6 Enzyme Production

Both submerged fermentation (SF) and solid state fermentation (SSF) are employed for production of enzymes. But SSF is a better method than SF for production of enzymes. Various enzymes have been produced by fermenting food processing waste. Invertase enzyme by fermenting sauerkraut waste with the help of *Canidida utilis* has been produced. This enzyme is widely used in the food processing industry. Subsequently, fungal amylase by using baked bean waste has been produced. Enzymes like cellulase and xylanase are produced by fermenting apple pomace, using *Trichoderma viridae* and *Aspergillus* sp. Pectinase is another enzyme which is produced from wastes like apple pomace.

Table 3.3: Food processing waste used as SCP/animal feed after microbial fermentation

Waste	Microorganisms utilised
Apple pomace	*Saccharomyces cerevisiae* *Candida utilis* *Torula utilis* *Aspergillus niger*
Corn cob	*Aspergillus niger*
Dried citrus peel	*Aspergillus niger*
Fodder beets	*Saccharomyces cerevisiae*
Orange peel and grape stalks	*Pleurotus ostreatus* *Agrocybe aegirata* *Armillarialla mellea*
Sugarcane bagasse	*Polyporus* sp. *Pleurotus* *Trichoderma*
Sugar beet pulp	*Trichoderma reesei* *Tricoderma viridae* *Fusarium oxysporum*

3.6.7 Citric Acid

Citric acid is being produced by fermenting brewery waste with *Aspergillus niger*. Apple pomace is a potential source of citric acid when fermented with *Aspergillus niger* by SSF on various substrates like pineapple juice, molasses, sweet potatoes residue, sugar cane bagasse impregnated with pineapple juice, mandarin orange waste, apple pomace, grape pomace. While production of citric acid by fermenting apple pomace, addition of methanol to the medium increases the yield of citric acid.

3.7 SINGLE CELL PROTEINS

In developing countries like India, deficiency of proteins leads to mal-nutrition. It has necessitated to explore new non-conventional resources of protein production. Amongst the various processes used to supply protein are those based on the microbial growth and microbial biomass especially using the waste material (Table 3.3). Microbial cells used as proteins as single cell protein (SCP) and can be used as protein supplement for feed or food. A number of micro-organisms like yeast, fungi, algae and bacteria can be employed production of SCP and each of them has its advantage and disadvantages. The micro-organism in turn use these substances as starting materials for fermentation and SCP production by assimilation.

The SCP however, is not without limitations also such as high nucleic acids which are metabolized to uric acid and can give rise to articular gout in human beings. Secondly, human being can eat a maximum of 2.0g SCP/kg

body weight/day in their diet. To overcome the nucleic acid levels in SCP various methods have been tried but with a variable success. The success of SCP depends upon economics of SCP production.

3.8 MICROBIAL FERMENTATION FOR UTILIZATION OF WASTE

With the advent of post-harvest technology, the fruits and vegetables are processed for the production of various products. The processing of fruits and vegetables in this way generate a large quantity of bio-degradable waste. The waste from processing of fruits and vegetables include water and various organic substances e.g. simple and complex polysaccharides (Sugars, starch, pectin, etc.), vitamins and minerals. The large quantities of waste generated in this way leads to environmental pollution. In today's environment conscious society, there are regulatory laws for the discharge of industrial effluent under the water conservation and control of Pollution Act 1974 and Environmental Protection Act, 1986 and these are mandatory for the processing industries. The waste from the processing industries can either be disposed-off after necessary treatment as per the directions of the pollution control agencies or it can be utilized by applying suitable technologies as illustrated in Table 3.4.

Table 3.4: Microbial utilization of food processing industry waste

Sl.	Products	Waste
1.	Ethanol	Citrus industry waste, apple pomace, peach waste, cashew apple pomace, pineapple waste, pear cutting.
2.	Biogas	Waste from fruit and vegetable industry as a whole, fermentation (wine and beer) waste.
3.	Single Cell	Apple pomace, peach waste, cashew apple proteins pomace, citrus waste extract, molasses, potato peels, cabbage waste.
4.	Cider, beer and vinegar	Apple pomace.
5.	Pectin	Citrus waste, apple pomace.
6.	Citric acid	Apple pomace.
7.	Baker's yeasts/ industrial yeast	Waste from wine, beer and distillary.
8.	Colour	Apple pomace.
9.	Flavours/Xanthan gum	Fruits and vegetable waste, citrus waste
10.	Animal feed	Apple pomace, peach waste, potato industry waste, olive processing waste.

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, toddy, vermouth, cider, beer and perry.

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2. What is lagering, pitching and draft beer?

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3. Name the yeast used in beer fermentation.

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4. What is role of boiling wort?

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5. Give various steps for wine and beer production.

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6. What is vinegar and SCP?

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7. Name the microorganisms and their sequence in lactic acid fermentation of cabbage.

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8. Name the processes used in vinegar preparation.

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9. Which is commercially available single cell protein source?

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10. Classify enzymes and their role in industry?

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11. Differentiate SF and SSF fermentation.

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12. Name the microorganisms associated with production of following products: Sauerkraut, Beer, Wine, Organic acid (acetic acid) and SCP.

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13. Define batch and continuous fermentation.

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14. Why yeast is preferred as a single cell protein compared to bacteria and algae?

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15. What are different types of fermenters? Enlist the same.

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16. Write 2-3 lines about the following:

Sauerkraut, Kimchi, vinegar

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3.9 LET US SUM UP

Fermentation is an ancient practice, carried out by natural or inoculated microflora. Several microorganisms are important as these are employed to produce the fermented food, additives and products of industrial significance. These include yeast, bacteria and fungi. Fermentation could be classified as solid state fermentation, submerged fermentation or extractive fermentation. Depending upon the mode of operation fermentation could be batch, fed batch or continuous type. At the industrial scale, the fermentationis carried outin the vessel called fermentor or bio reactors. Depending upon the type of fermentation or product, the type of fermentoris employed. Food fermentations include those to produce wine, beer, brandy, whisky, pickles, sauerkraut, kimchi, vinegar, yoghurt additives like citric acid, lactic acid, enzymes, ethanol, single cell proteins being produced commercially using microbial process.

3.10 KEY WORDS

Fermentation : was used for the production of wine but at present it encompasses the foods made by the application of microorganisms including lactic acid bacteria (LAB).

Bakers' yeast : The strains of *Saccharomyces uvarum* used to make bread.

Wine yeast : *S.* cerevisiae *var. ellipsoidus.*

Distillers yeast : High alcohol yielding strains of *S. cerevisiae* var *ellipsoidu* used to higher alcoholic beverages.

Controlled "starter" Culture : pure as well as mixed cultures of microorganisms are responsible for conducting fermentations, employed in the manufacture of certain food and dairy products such as fermented milk, butter, cheese, bread, malt beverages.

Enzymes : Enzymes are biological catalysts possessing efficiency and specificity and are mostly protein in nature.

Solid state fermentation : Fermentation processes which take place in the absence or near absence of free water in the substrate are termed as solid state fermentation (SSF).

Submerged fermentation : Fermentation processes which take place in the presence of free water in the substrate are termed as sub-merged fermentation.

Fermenter : The industrial usage of micro-organisms often requires that they be grown in large vessels containing considerable quantities of nutritive media. These vessels are commonly called fermenteors.

Wine : The term 'wine' is applied to the product made by the alcoholic fermentation of grape or grape juice. But any fleshy fruit or flower in the new world may be employed.

Beer : Beer is an alcoholic beverage prepared from barely or other cereals.

Vinegar : The word vinegar is derived from two French words, *vin* and *aigre* meaning sour wine but the term vinegar is used to denote a condiment prepared from various sugar and starch containing materials by alcoholic and subsequent, acetic fermentation.

Acetous fermentation : or acetic acid fermentation which is oxidative fermentation carried out by acetic acid bacteria viz. *Acetobacter aceti*.

Lactic acid bacteria (LAB) : These are the microorganisms that are obligate producing lactate from sugars as the main end product, besides producing inhibitory substances to other microorganisms.

Sauerkraut : It is the product of characteristic flavour, obtained by lactic fermentation of cabbage in the presence of 2-3% of salt.

Kimchi : It is a group of fermented vegetable foods of Korea with cabbage or radish as the main ingredient.

Single cell protein : The microbial biomass used as protein supplement for feed or food is called as single cell protein (SCP).

3.11 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

1. Your answer should include the following points:

   Wine: It is the product made by the alcoholic fermentation of grape or other fruit or flower.

   Cider: Cider is a low alcoholic beverage from apple juice.

   Vermouth: Wine flavoured with a characteristic mixture of herbs and spices, with an aromatic flavour and odour while others a bitter flavour, sweet or dry with alcohol content of 15 to 21%.

   Toddy: Sweet alcoholic drink, having alcohol content of 4-6%, made by the fermentation of sap from coconut palm.

2. Your answer should include the following points:

   Lagering: It is the process of storage of young, green or draft beer in vats at about $O^{o}C$ for several weeks to several months, to make it clear and mellow.

   Pitching: It is the process of inoculation of yeast culture in the wort.

   Draft beer: The freshly prepared beer (not matured) is called draft beer.

3. Your answer should include the following points:

   The answer is *Saccharomyces cerevisiae* var *carlbergenesis.*

4. Your answer should include the following points:

   The boiling concentrates, sterilizes the wort and inactivates the enzymes.

5. Your answer should include the following points:

   Various steps are involved in beer production:

   Malting
   Mashing
   Wort boiling
   Fermentation
   Aging or Maturing
   Finishing
   Table wine
   Juice Extraction
   Must preparation
   Fermentation
   Siphoning/racking
   Maturation
   Clarification
   Blending
   Pasteurization

6. Your answer should include the following points:

   Vinegar: Vinegar is the product obtained by acetic acid fermentation of ethanolic liquid of any fruit and contains 3.75 %w/w acetic acid. It is prepared by alcoholic fermentation of fruit juice.

   SCP: Single cell proteins (SCP) are the microbial cells used as a source of proteins. To produce single cell proteins, suitable microorganism and medium is selected. The optimum conditions of growth are also determined and the same are given during the fermentation. The grown biomass is then harvested and the cells are used as a source of proteins.

7. Your answer should include the following points:

   First of all *Pediococus* comes, then *Streptococcus* followed by *Lactobacillus in* cabbage fermentation.

8. Your answer should include the following points:

   Slow process: This process takes long period wherein the juice is allowed to undergo alcoholic acetic fermentations. It takes about 5-6 months to complete the fermentation to form the vinegar from the juice.

   Quick process: In quick processes like generator process alcoholic liquid is kept in motion and this process is applied mostly to the production of vinegar from spirit (alcohol). The alcoholic fermented liquors is well supplemented with food for the vinegar bacteria, such as a combination of organic and inorganic compounds. The process needs additional supply of oxygen.

9. Your answer should include the following points:

   Spirullina which is used as a SCP commercially.

10. Your answer should include the following points:

    All the enzymes have been classified into six classes.

    Class 1 – Oxido-reductase e.g. dehydrogenases, peroxidases.

    Class 2 – Transferase

    Class 3 – Hydrolase

    Class 4 – Lyase

    Class 5 – Isomerase

    Class 6 – Ligase

    Uses of enzymes in food processing: Pectinase enzyme are used in clarification of juices (apple juice, guava juice), lemon juice etc. Pectinase enzyme result in softening of apple fruit, tomatoes, peaches, avocadoes and thereby resulted in increase in yield of juice and pulp during processing. Pectinase enzyme result in easy extraction of juice from fruits. Proteases resulted in clarification and removal of cloudiness in beer and wine. Glucose oxidase enzyme is used in removal of glucose from egg white and thereby, improve, the colour of dehydrated egg powder. Pectinase with cellulase enzymes are also used for extraction of oil from oil containing fruits. Beer cloudiness can be removed by use of proteases e.g. papain. Enzyme diastase resulted in conversion of starch to sugars during beer preparation.

11. Your answer should include the following points:

    The submerged fermentation (Smf) makes use of free liquid while in that of solid state fermentation (SSF) no free water is available.

12. Your answer should include the following points:

    The microorganisms used for the products are listed as below:

    Sauerkraut: Lactic acid bacteria

    Beer: *Saccharomyces cerevisiae* var *carbergenesis*

    Wine: *Saccharomyces cerevisiae* var *ellipsoideus*

    Organic acid (Acetic Acid): *Acetobacter aceti*

    SCP: *Spirullina, Saccharomyces cerevisiae, Candida utilis*

13. Your answer should include the following points:

    Batch fermentation: Here the starter culture is added to the medium and the product is withdrawn after completion of fermentation.

    Continuous fermentation: Here the substrate is continuously fed to the fermenter and the product is also withdrawn continuously.

14. Your answer should include the following points:

    1. Yeasts have nutritive value especially proteins and vitamins.
    2. Able to grow on a variety of carbon and nitrogen source.

3. Has faster growth and high yield, ability to grow at low pH.
4. Can grow on a large number of waste including that from processing industries.

15. Your answer should include the following points:

Types of Fermentors:

1. Shake flasks and bottles
2. Stirred tanks
3. Airlift fermenters
4. Tower fermenter
5. Rotating disc fermenter
6. Fixed bed fermenter
7. Fluidized bed fermenter

16. Your answer should include the following points:

Sauerkraut: It is the clean, sound product of characteristic flavour, obtained by full fermentation, chiefly lactic of properly prepared and shredded cabbage in the presence of not less than 2% nor more than 3% of salt.

Kimchi: It is a group of fermented vegetable foods of Korea with cabbage or radish as the main ingredient with or without cucumbers.

Vinegar: The word vinegar is derived from two French words,*vin* and *aigre* meaning sour wine but the term vinegar is used to denote a condiment prepared from various sugar and starch containing materials by alcoholic and subsequent, acetic fermentation.

3.12 SOME USEFUL BOOKS

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UNIT 4 SPOILAGE AND ASSOCIATED CHEMICAL/PHYSICAL CHANGES IN FOOD

Structure

4.0 OBJECTIVES

After reading this unit you should be able to:

- state the meaning of spoilage;
- principle of preservation of food;
- discuss various causes of spoilage;
- describe different types of spoilages; and
- preventive measures which should be taken.

4.1 INTRODUCTION

We all know that food is the basic necessity of all the living entities. Needless to say that such a commodity has to be absolutely safe and of highest possible quality especially free from toxins and spoilage. A food is said to be spoiled if it has been damaged or injured making it unsuitable for human use. "A product is fit as a food if a discriminating consumer, knowing the story of its production and seeing the material itself, will eat it, and conversely, the same product is spoiled when such an examiner refuses it as a food". All of us would agree that a food is spoiled if it is not harvested at proper maturity, is contaminated with dirt, handled by dirty or diseased person, is fertilized with sewage and has objectionable changes due to the activity of microorganisms or action of enzymes of the food. The major causes of spoilage are: the microorganisms or their enzymes, the native enzymes of food, rodents, environmental factors and purely chemical reactions.

It must be admitted that despite of the improvement in the methods of production, handling and processing, the microbiological quality still remains the most important factor. This aspect assumes significance from toxin production, spoilage of fresh and processed products and quality control and as sanitation indicators in a processing unit. Microbial quality is also on the top of the different hazards which are associated with the safety of food for consumption by human beings. Various fruits, vegetables and their products may be spoiled by one or more factors like unsuitable packaging, chemical changes or action of microorganisms, tissue enzymes, insects, rodents or improper methods of processing, under processing, etc. Different spoilage causing agents for various fruit and vegetable products, their prevention and health hazards associated with spoilage are also discussed here.

4.2 PRINCIPLES OF FOOD PRESERVATION

In accomplishing the preservation of food by various methods, three main principles are involved:

I. Delay or prevention of microbial decomposition of food
II. Delay or prevention of self-decomposition of food
III. Prevention of damage caused by insects, rodents, birds, mechanical causes etc.

Various principles and sub-principles of preservation of food are summarized in Table 4.1. First principle of food preservation is based mainly on the following considerations:

- By delaying the microbial decomposition of food
- By preventing the microbial decomposition of food

Most of the methods of food preservation depend not only on the destruction or removal of microorganisms but also on the delay in the initiation of their growth, and hindrance to growth once it has begun. Knowledge of growth curve of microorganisms is very helpful for developing the appropriate technique to delay the microbial decomposition of the food.

Table 4.1: Detailed principles of preservation

I. Delay or prevention of microbial decomposition of food
i) by keeping out microorganisms (asepsis).
ii) by removal of microorganism.
iii) by hindering the growth and activity of microorganisms.
iv) by killing the microorganisms.
II. Delay or prevention of self decomposition of food
i) by inactivation of food enzymes.
ii) by delay or prevention of purely chemical reactions.
III. Prevention of damage to food caused by insects, rodents, birds and mechanical causes

4.3 CLASSIFICATION OF FOODS BASED ON PERISHABILITY

1. *Perishable foods:* The foods which spoil readily unless special preservation methods are used such as meat, fish, most of fruits and vegetables, egg, poultry and milk etc.
2. *Semi-perishable foods:* Semi-perishable foods like waxed potatoes and some varieties of apple, if handled and stored properly, shall remain unspoiled for a fairly long period.
3. *Non-perishable foods:* These foods do not spoil until and unless they are handled carelessly. Such foods are also called as stable foods such as cereals, sugar.

4.4 FACTORS GOVERING SPOILAGE

Since microorganisms are the main spoilage causing factors, major emphasis remains on the factors related to microbial spoilage of the foods. Here bacteria cause most of the problems since these are not killed at ordinary temperatures. The yeasts and molds have low resistance to heat (processing temperatures). The main factors responsible for such spoilage are described here.

4.4.1 Number and Kind of Microorganisms

The initial number of microorganisms present on the food has direct relationship with its spoilage. More the number of microorganisms present, rapid is the spoilage. The effect of initial number of spores on time required to kill them is shown in Table 4.2. The heat resistance of bacteria involved in food poisoning is of major concern from public health view point. Usually, only one type of microorganisms will be there because of the particular environmental conditions involved. However, contamination may increase the number as well as new kinds of microorganims.

Table 4.2: Effect of initial number of spores on time required to kill them

Initial concentration of cells, number/ml	Thermal death time, or time required to kill all spores min. at 121°C
50,000	14
5,000	10
500	9
50	8

In addition to destruction or removal of microorganisms, the delay in the initiation of growth also prevents microbial spoilage. This is done by keeping the microorganisms in lag phase as long as possible. Once the microorganisms enter the log phase, it is very difficult to control them. A typical growth curve of microorganism is shown in Figure 4.1.

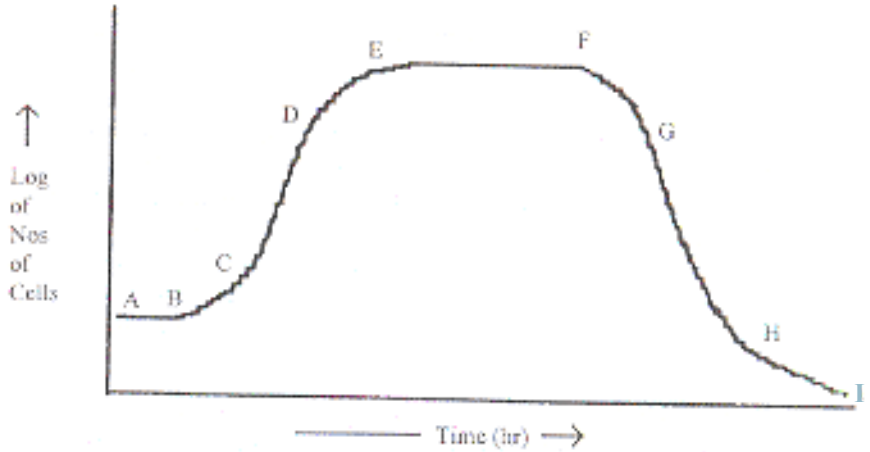

Figure 4.1: Growth curve of microorganism. A to B lag phase, B to C positive acceleration phase, C to D logarithmic phase, D to E negative acceleration phase, E to F stationary phase, F to G accelerated death phase, G to H death phase, H to I survival phase

4.4.2 Suitability of Temperature

Different microorganisms grow at different temperatures. Psychrophiles have affinity for low temperatures (8-10^{o}C), mesophiles grow best at medium temperatures (25-40^{o}C), while thermophiles appear at higher temperatures (50-55^{o}C). A large number of microorganisms grows at mesophilic temperature therefore processed foods are immediately cooled to arrest microbial activity / spoilage of foods. So, storage temperature is very important in relation to microbial growth and hence, the spoilage behaviour of foods.

Different types of microorganisms require different times to kill their cells or spores. The time required to kill all the spores of flat sour bacteria (*Bacillus stearothermophilus)* in relation to temperature is shown in Table 4.3.

Table 4.3: Effect of temperature of heating on the time needed to kill spores of flat sour bacteria

Temperature (^{o}C)	Thermal death time or time to destroy all spores, min.
100	1200
105	600
110	190
115	70
120	19
125	7
130	3
135	1

Source: Adapted from Frazier and Westhoff (1996).

4.4.3 Suitability of food

Different microorganisms prefer different kinds of foods. Some grow best on proteinacious foods, others on starchy or fatty foods. The physical state of the food whether heated, frozen, moistened or dried, also has an important

influence on the spoilage it will undergo. The moisture content also influence the type of microorganisms in the foods since the requirements of moisture for their growth are different. Bacteria, yeast and molds have different moisture requirements in terms of water activity (Figure 4.2).

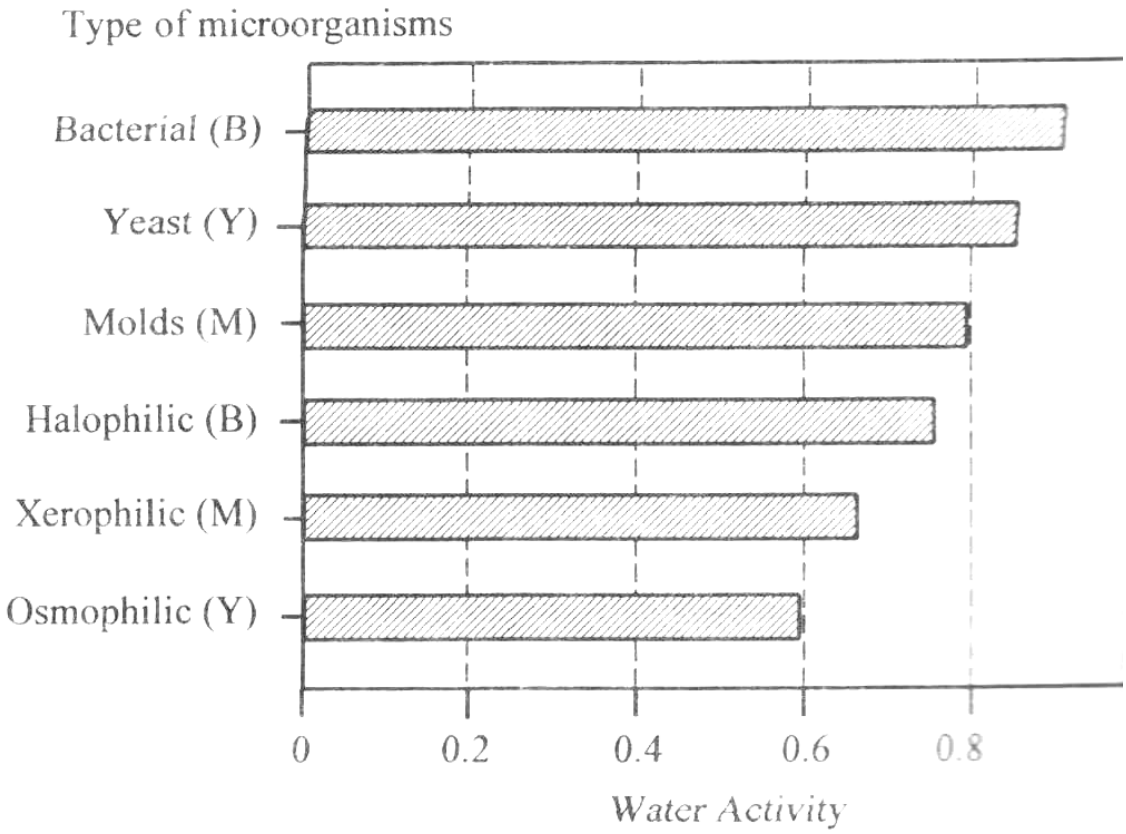

Figure 4.2: Water activity of different microorganisms

4.4.4 pH of Food

The pH of the food influences the kind and growth of microorganisms. The composition of the vegetable, its pH and moisture contents affect their type of spoilage. As a general rule, foods having pH<4.5 (acid foods) do not require heat processing (particularly cooking under pressure), but those with pH > 4.5 (low acid foods) always require processing under pressure.It is because of the reason that thermophilic bacteria may not be killed at normal temperatures as most bacteria thrive best at pH of 4-7.5, while the yeasts and molds require a pH of 2.5-8.0 and 1.5-8.5 for their growth.

4.4.5 Presence of Air

Aerobic or anaerobic microorganisms can be found in the spoiled fruit and vegetable products, depending upon the presence or absence of air (oxygen) in the container or package.

4.5 CHEMICAL AND PHYSICAL CHANGES ASSOCIATED WITH FOOD SPOILAGE

Changes in nitrogeneous organic compounds: Most of the nitrogen in the food present in the form of proteins which are hydrolysed by enzymes to produce amino acids. The anaerobic decomposition of the protein, peptide or amino

acid results in the production of obnoxious odour which is called as putrefaction. When the microorganisms act on amino acid they deaminate or decarboxylate.

E. coli produces glyoxylic acid, acetic acid and ammonia from glycine and from serine it produces pyruvic acid and ammonia. Alanine is degraded into α-keto acid, ammonia and carbon dioxide by *E. coli*; acetic acid, ammonia, and CO_2 by *Psuedomonas*; and propionic acid, acetic acid, ammonia and CO_2 by. *Clostridium nigrificans*. Other nitrogenous compounds like amide, urea, guianidine and creatine, etc. are also decomposed to ammonia, carbon dioxide and other products.

Changes in Carbohydrates: Carbohydrates are preferred by the microorganisms as energy yielding foods. They hydrolyse the polysaccharides to monosaccharides before utilization such as to glucose which is then, oxidized to CO_2 and H_2O. Anaerobically, these undergo decomposition involving one or more types of fermentation.

- Alcoholic fermentation by yeast with ethanol and CO_2 as products.
- Lactic fermentation by homofermentative lactic acid bacteria with lactic acid or by heterofermentative lactic acid bacteria with lactic acid, acetic acid, ethanol, glycerol and CO_2 as chief products.
- Coliform type of fermentation by coliform bacteria with lactic acid, formic acid, ethanol, CO_2, hydrogen and perhaps aceton and butanediol as likely products.
- Propionic acid fermentation by propionoic bacteria producing propionic acid, acetic and succinic acid and CO_2.
- Butyric- butyric isopropyl fermentation by anaerobic bacteria producing butyric acid, acetic acid, CO_2, H_2 and in some cases, butylenes glycol, butanol and 2-propanol.

They are present as salts and are oxidized by the microorganisms to carbonate and cause the food medium to become alkaline. Organic acid aerobically are oxidized to carbon dioxide and water as is done by the film yeast.

Changes in other compounds: Other compounds also undergo changes as detailed here:

- Ethyl alcohol is oxidized to acetic acid.
- Glycoside is hydrolysed to sugars.
- Acetaldehyde is oxidized to acetic acid or reduced to ethanol.
- Protopectin are acted upon by pectinesterase – pectic acid + methanol (water soluble) by hydrolysis of methyl ester. Polygalacturonases destroys the linkage between galactouronic acid unit of pectin or pectic acid to yield smaller chain and ultimately, free D-galacturonic acid, which may be degraded to simple sugar.

Changes in Lipids: Fats present in the media are hydrolysed by lipase into glycerol and fatty acid.Phospholipids may be degraded to their constituents phosphate, glycerol, fatty acid, and nitrogenous base e.g. chlorine.

One or more such changes can be produced in the food undergoing spoilage. The physical and sensory qualities of the food also undergo changes, thus making the product unfit for human consumption.

4.6 MICROBIOLOGY OF FRESH FRUITS, VEGETABLES AND THEIR PRODUCTS

4.6.1 Spoilage of Fruits and Vegetables

In general, fruits and vegetables are bulky, easily damaged mechanically, consist largely of water which is readily lost and above all, are living entities and must be kept so for their longivity. Thus, they are sensitive to their environment viz., temperature, level of oxygen, carbon dioxide and ethylene etc. Spoilage of fresh fruits and vegetables usually occurs during storage and transport and while waiting to be processed into various products. These also get contaminated with spoilage organsims either from each other or when they are laid into the baskets, lugs, boxes etc. during harvesting. Mechanical injuries during transportation further aggravate the deterioration process. The decay of perishables may occur due to the physical factors, action of their own hydrolytic enzymes or microbial contaminants etc as discussed earlier. Since fruits and vegetables after picking are alive for certain time thus, are sensitive to their environment, their rate of metabolism is temperature dependent and they may be damaged by heat or cold or even by levels of different gases in the atmosphere. Oxygen is taken in during respiration and CO_2 heat and water vapours are given-off. As fruits or vegetables are detached from the mother plant, the continuity of the flow of sap is totally disrupted but the respiration and water loss continues leading to exhaustion of food reserve and moisture. The irreparable losses are caused leading to deterioration and eventually spoilage. Spoilage is mainly of 2 types: Abiotic spoilage and Biotic spoilage.

Abiotic spoilage: It is due to the different physical (wilting, caking and melting etc.) and chemical changes in the product (hydrolytic action of enzymes, oxidation of fats, putrefaction of proteins, browning reaction between proteins and sugars). Temperature control is the major factor to provide longevity to the fruits and vegetables.

Biotic spoilage: This includes the microbial action associated with bacteria, yeasts and molds on vegetables and fruits and the normal processes of aging. The species of microorganisms causing food spoilage largely depend upon different factors e.g. kind and variety of fruits/vegetables, environmental condition e.g. storage, temperature, relative humidity of the atmosphere and various gas contents of the atmosphere etc. There are two types of microbial spoilage: (a) Spoilage caused by plant pathogens which attack various parts of the plant used as foods, (b) Spoilage caused by saprophytes. The most common and general type of spoilage in fruits and vegetables are mildew are listed in Table 4.4. Dry rots often lead to darkening and discoloring, and hardening of the surface of vegetables and fruits. In microbial spoilage, the vegetables often develop water soaked musky areas while the fruits generally have brown or white colored patches.

Table 4.4: The chief market diseases of some vegetables and fruits

Item	Market diseases
Onions	Bacterial soft rot, black mold rot, gray mold rot
Garlic	Bacterial soft rot, black mold rot
Green beans	Bacterial soft rot, mold rot, *Rhizopus*
Carrots	Bacterial soft rot, black rot, *Fusarium* rot, gray mold rot, watery soft rot
Beets	Bacterial soft rot, black rot, blue mold rot, *Fusarium* rot
Lemons	*Alternaria* rot, anthracnose, blue mold rots, stem-end rots
Peaches	*Alternaria* (or green mold rot), gray mold rot, black mold rot
Apricots	Blue mold rot, brown rot, *Cladosporium* rot, *Rhizopus* rot
Bananas	*Anthracnose, Fusarium, Gleoporium, Pestalozia*
Grapes	Black mold rot, gray mold rot, *Rhizopus* rot, blue mold rot
Strawberries	Gray mold rot, leather rot (*Phytophthora cactorum*) *Rhizopus* rot
Pears	Black rot, blue mold rot, brown rot, gray mold, *Rhizopus* rot
Potatoes	*Fusarium* tuber rot, bacterial ring rot, bacterial soft rot
Cucumber	*Rhizopus* soft rot, bacterial soft rot, blue mold rot, gray mold rot
Cabbage	Bacterial soft rot, gray mold rot, black rot, watery soft rot
Cauliflower	Bacterial soft rot, gray mold rot, black rot, watery soft rot
Tomatoes	*Alternaria* rot, bacterial canker, bacterial spot, gray mold rot, green mold rot, *Rhizopus* rot

The composition of the fruit/vegetable, its pH and moisture content affect their type of spoilage. Moisture content is usually expressed in terms of water activity ‘aw’. Various microorganisms have different requirements for moisture level (Figure 4.2). Amongst the microorganisms, spoilage can be caused by bacteria, molds/yeasts etc. depending upon the pH of food.

Bacteria: Various groups of bacteria can attack different fruits and vegetables, depending upon their composition such as lactic acid bacteria, acetic acid bacteria, coliform bacteria and sporeforming bacteria. The food can be preserved for longer time by prolonging the lag phase. This can be obtained by avoiding the contamination of the food and turning the environmental conditions e.g. temperature, moisture and pH unfavourable for the growth of contaminants (microorganism). Thus, by lowering the storage temperature of the fruits/vegetables, filling up the storage chamber with the inert gases will definitely lead to longer shelf- life of the vegetables and fruits. pH is another important factor governing the bacterial growth which range between pH 4-8. Growth rate is lowered by a decrease in pH.

Yeasts: Yeasts are widely found in the environment. The yeast growth depends largely upon the nature of fruit product. These are generally fermentative in nature.

Molds: Molds are frequently associated with food products. Some of the molds secrete toxic compounds (mycotoxins) like aflatoxins, patulin etc. Aflatoxin has been detected in dried figs and fig paste while patulin is the most common mycotoxin detected in the processed fruits. The mold *Penicillium expansum* which causes apple rot and some other molds produce patulin. The mycotoxins are deleterious to various animals and presumably the human beings also.

4.7 SPOILAGE OF PROCESSED FRUIT AND VEGETABLE PRODUCTS

The spoilage of processed fruit and vegetable products is also of two types: Abiotic spoilage and Biotic spoilage.

a) **Abiotic spoilage:** It is due to the different physical and chemical changes in the product viz. putrification of proteins, browning reactions between sugars and proteins and the physical changes of colour, caking and melting etc. The temperature and humidity are the main factors responsible for causing this type of spoilage. There is a relationship between moisture content and relative humidity with respect to different causes of spoilage in food products.

b) **Biotic spoilage:** It includes the actions associated with microorganisms, damage caused by insects, rodents etc.

Manifestation of spoilage: The microbial deterioration of a processed food product usually is manifested by alterations in the appearance, texture, colour, odour, flavour or slime formation. The appearance includes colour changes, visible growth of microorganisms, formation of pockets of gas or swelling of cans and microbial growth especially that of molds on the surface of food process (Plate 4.1). As some food products deteriorate, they tend to become soft or mushy. Degradation of foods results in the formation of compounds which have odours and flavours different from those of the fresh food.

Plate 4.1: Different spoiled products from fruits and vegetables. 1) Mold growth on pickle, 2) Mold growth on jam, 3) Mold growth on juice, 4) Mold growth on tomato crush, 5) Puffed can

Spoilage of Canned Fruits and Vegetables

Like other foods, canned fruit and vegetable products are also liable for spoilage for various reasons. Number of microorganisms surviving the heat process, storage temperature, suitability of the canned food to support the growth of the microorganisms, pH of the food, oxygen tension etc. may affect the spoilage of canned foods. Broadly, there are four causes for the spoilage of canned fruit and vegetable products viz., microbiological, physical, chemical and miscellaneous.

Microbiological spoilage: The spoilage caused by the growth of different kinds of microorganisms may be affected by under processing, inadequate cooling, infection resulting from leakage and pre-processing spoilage.

Under processing: Insufficient or improper heat treatment may result in survival of certain microorganisms causing spoilage of food product during subsequent storage. Some lot or a part of the lot may remain under processed or not processed at all (gross under processing) by mistake. As a general rule, only one type of microorganisms (bacteria or yeast or mold) are involved in such spoilage. Most of these are facultative anaerobes unusually heat resistant spore formers. Faulty operation during processing may also cause under processing. This type of spoilage can be avoided by ensuring proper heat transfer, proper retorting and proper stacking of cans in the retort.

Inadequate cooling: Immediately after processing, the cans are cooled using cold water. It gives a sort of shock to the surviving microorganisms and kills them. It also checks overcooking and hence, saves the food from textural disintegration. If cooling is not proper, spoilage can occur. In addition, the cans are cooled down (using fans also) upto 35-38^{o}C only to allow the water to evaporate readily from the can surface. It will check the rusting of cans during storage, which may otherwise lead to some spoilage. It can be avoided by proper cooling after processing.

Infection due to leakage: Post-processing contamination may take place if there is leakage in the cans due to faulty seam, faulty lock seam or pinholes due to corrosion from inside of the can or rusting of can from outside. Here all the types of microorganisms can be present in the food. Lot of oxygen can enter into the can. Contents of such type of cans are not suitable for consumption. To avoid such kinds of spoilage, seam tests should be carried out regularly while in operation, proper hygiene should be maintained and handling should be proper. The cooling water should be chlorinated using 5-7 ppm of chlorine.

Pre-process spoilage: If the raw material is already heavily contaminated many microorganims may survive heat treatment and also the finished product may not be of desirable flavour or quality. It may also be due to faulty procedure during washing (of raw materials, cans and equipment) blanching and filling of cans. Many respiratory gases can develop causing swelling of cans during storage. To avoid such type of spoilage, proper testing of raw materials, proper washing of raw material as well as equipment, chlorination of water and proper sterilization of cans should be practiced.

Thermophillic spore forming anaerobic spoilage: The microbiological spoilage of canned fruits and vegetables can be of different types.

Flat sour: It derives its name from the fact that the ends of the can of food remain flat but the contents become sour. It is mostly found in non-acid foods like canned vegetables by the action of microorganisms (flat sour bacteria). So it cannot be detected without opening of can and culturing the microorganisms. *Bacillus stearothermophilus* and *Bacillus coagulans* are the thermophilic spore-forming bacteria responsible for flat sour type of spoilage. The latter are found in tomato juices. The immediate source of the flat sour bacteria is usually the plant equipment besides sugar, starch or soil.

TA spoilage: It is the short name for spoilage caused by thermophilic anaerobes which do not produce H_2S e.g. *Clostridium thermosaccharolyticum*. It is a sugar-splitting obligate thermophilic, sporeforming anaerobe which produces CO_2 and H_2, causing the swell and even bursting of cans. The spoiled food usually have sour odour and the source of contamination could be the plant equipment, sugar, starch and soil similar to that of flat source.

Sulphide or Sulphur Stinker spoilage: Such spoilage is caused by *Clostridium nigrificans*, mostly found in low acid foods like peas and corn. The spores of this bacterium are considerably less heat resistant and hence, their appearance in canned foods is indicative of gross under processing. H_2S can be detected by its characteristic odour on opening the can and the organism can also be detected in the form of black (FeS) colonies formed on iron sulphite agar at 55^oC.In case of peas, it is difficult to detect any marked discolouration. The source of this organism includes sugar, starch, soil, manure and the plant equipment.

Mesophilic spoilages: Some species of *Bacillus* and *Clostridium*, non-spore forming bacteria and even yeasts or molds may spoil the under-processed canned foods. The spore forming mesophilic thermoduric species of bacteria include *C. pasteurianum, C. butyricum, C. botulinum, C. sporogenes, B. sublitis, B. polymyxa,* etc. The non-spore forming mesophilic bacteria involved in the spoilage of tomato products, pears and some other fruits were found to be *Lactobacillus* and *Leuconostoc*. Other such genera may be *Pseudomonas*,

Micrococcus, *Flavobacterium* etc. which may come from water and leaks in the cans.

Spoilage by yeasts: The yeasts have been found to spoil canned fruits, jams, jellies, fruit juices, etc. underleakage or under-processing conditions. Film yeasts like *Candida, Pichia*, *Hansenula* can grow on acid products like saurkraut and pickles osmophilic yeasts like *Saccharomyces rouxi*, *S. mellis* can spoil dry fruits, concentrated fruit juices and honey etc. Salt tolerant yeasts like *Torulopsis* and *Brettanomyces* can grow in brine solution.

Spoilage by molds: The molds are the common spoilage organisms of home canned foods like jams, jellies, marmalades. The common one are *Aspergillus, Penicillium, Byssochlamys fulva* etc.

Spoilage by physical causes: Some physical deformation of the container can lead to spoilage. Faulty technique in operation, under exhausting, over filling and panelling or buckling of the cans can cause spoilage of processed food products.

Faulty technique in operation: Just after retorting, if the pressure is released at once instead of slow release, distortion of can body can occur. Joints or seams may be distorted resulting in leakage. To avoid such kind of spoilage, the pressure in the retort should be released slowly after retorting and standard iron plates should be used for cans.

Under-exhausting: If the air entrapped in the tissues of canned fruit and vegetables, and the filling medium is not expelled properly, adequate vacuum may not develop which may consequently, impair the quality and appearance of the product.

Over-filling: Over-filling of cans does not allow proper vacuum formation after processing. It may also lead to flipper or springer type of spoilage. The proper filling is essential to avoid this defect.

Panelling or buckling: It occurs in case of big sized cans. If there is very high vacuum inside the can, atmospheric pressure can struck or force the can inwards resulting in leakage type of spoilage. To check such spoilage, proper vacuum should be created in the cans carefully.

Chemical spoilage: It includes reactions among ingredients, reactions between can and ingredients, hydrogen swell etc. Mainly, it is due to H_2S production, presence of oxygen, acids etc. H_2S is formed by the action of SO_2 (added through sugar or by decomposition of proteins) and H_2 formed by fruit acid acting on tin plate. If there is sufficient vacuum, H_2 is absorbed after storage for long time. Low acid foods have more H_2 swells. Therefore, pH of the food has important role in checking such spoilages. If the pH is near 4.0, it is favourable for many chemical reactions. To avoid the spoilage, we can assure proper vacuum (by hot filling) in the can and also adjust safe pH of the food prior to canning. Rusting and corrosion and perforation of tin plates: After cooling the processed cans in water, if some water remain on the surface of cans, rusting can take place. Similarly, the hygroscopic nature of the labels can also add to the rusting. In case of acid foods, there are more chances of corrosion and perforation of tin plates. More the oxygen in the can, more is the corrosion. Corrosion is more at higher than at lower temperatures. To avoid corrosion and perforation of tin plates, proper exhausting should be done and cans should not be cooled below 35^oC in water or fans should be used to

evaporate water from the can body and non- hygroscopic in nature labels should be used and be stored at relatively low temperatures.

Metallic contamination: The tannins of raw materials or spices used react with exposed iron of the tin plate to form ferric tannate, a black product. Similarly, the SO_2 on reacting with H_2 forms H_2S that may further react with iron content of the can to form iron sulphide thus, causing spoilage of the processed food product. Also, on using the equipment made of copper or brass after sometime, in spite of thoroughly cleaning, small traces of copper oxide may remain there, which further form black copper sulphide on reacting with H_2S and discolour the product. To avoid such spoilage, proper exhausting and proper selection of equipment, thorough washing of the raw material as well as equipment.

External Appearance of Can

Flipper: A can with mild positive pressure is called a flipper. It may be an initial stage of swell or hydrogen swell but more frequently, it is due to over-filling or under exhausting, leakage or sealing at low temperature. The can ends remain flat but when the sides of can are struck with some hard structure or if the temperature of the contents is increased, bulging of ends take place.

Springer: A mild swell at one or both ends of a can is called a springer. One end may also remain permanently bulged and other flat. Pressure on the bulged end will bring it to normal but it will go to other end. Generally, the food in such cans remains fit for consumption. The reasons are similar as for flipper can.

Swells: In this case, both ends remain bulged. It may be a soft swell or hard swell. In case of soft swell, the ends are not so hard. On applying some pressure, the ends may go inward (normal) but do not remain normal on the removal of pressure. Obviously, in hard swell, can ends are rigidly hard and there is no effect of pressure except bursting or leakage of the can. The swell occurs due to the production of H_2 (formed by action of acids of food and tin plate), CO_2 or other gases (as a result of decomposition of contents by microorganisms) involving both thermophiles or mesophiles. The food is not fit for consumption and may even contain toxins produced by *Clostridium botulinum*.

Leaker: A very small leak may appear in the can due to faulty seam, faulty lock seam or pinholes resulting from corrosion from inside of the can or rusting of the can from outside.

Breather: Tiny leak in the can may allow air to pass back and forth into the can but not the microorganisms. The inside pressure of the can equals outside pressure. The contents may be spoiled due to rusting of can caused by oxygen in air passing through the tiny leak.

Buckled cans: Sometimes, the vacuum in big sized cans is so high that atmospheric pressure can strike the can body resulting in deformation of can leading to leakage of contents or contamination.

Spoilage of Fruit and Vegetable Juices

Fruit juice/squashes: The fruit juices are more spoiled by yeasts and molds than by bacteria since they have lower pH while vegetable juices are spoiled more by bacteria than yeasts and molds because of very high pH. If

the fruit and vegetable juices are not processed after extraction, they are spoiled because of enzymatic changes and microbial actions. Apple and grape juices are spoiled by bacteria if the temperature of storage goes above 25^{o}C. Molds can grow on the surface of fruit juices if exposed to air. Most fruit juices have sufficient sugar to favour the growth of yeasts. Deficiency of B group vitamins discourages some bacteria. Concentrates of fruit and vegetable juices favour the growth of yeasts and of acid bacteria and sugar tolerant (*Zygosacharomyces)* species because of increased acidity and sugar concentration. Fruit juice concentrates are fermented almost exclusively by *Saccharomyces rouxi*, *S. mellis*, *Torulopsis* and *Hansenula*. Typical fermentation products are ethanol and CO_2. Heat treatment during canning of these concentrates usually kills these microorganisms and freezing prevents the growth of such organisms. In fruit squashes, if presevatives are not added in proper concentration, some yeasts (e.g. *Zygosaccharomyces*) can spoil such products.

Spoilage of canned fruit juices: The acidity is the single most important factor affecting microbial spoilage of fruit juices. Most bacteria have an optimum pH near 6.8 but may grow at pH values ranging from 4-8. Yeasts and molds can grow at pH <2.

In canned foods, a pH of 4.5 is used as a borderline between acid and low acid foods, that is foods not requiring and those requiring respectively, the minimum botulinum cook (12 D). The typical spoilage flora of fruit juices is represented by some Clostridia, *Bacillus*, members of Enterobacteriaceae, lactic acid bacteria, *Acetobacteriaceae*, yeasts and molds. The spoilage is characterised by lowering of pH (0.2-0.4 units), development of very high volumes of hydrogen and CO_2, and strong cheesy (butyric) odour. Three types of Bacilli are usually involved in spoilage of fruit juices i.e. *B.coagulans* (flat sour), *B. macerans* and *B. polymyxa* (both by storage at temperature < 46^{o}C).

The spoilage by enterobacteriaceae is characterised by the production of lactic acid, acetic acid, formic acid, H_2 and CO_2. Lactic acid bacteria causing spoilage of fruit juices include *Lactobacillus, (L. plantarum, L. fermenti) Leuconostoc mesenteroides, Streptococcus viscosum*. Among the yeasts, the most often involved species in juice fermentation belong to *Saccharomyces, Torulopsis, Candida, Pichia, Hansenula* and *Hanseniaspora.*

Molds require O_2 for development and so usually do not grow at the surface of processed fruit products. However, *Byssochlamys fulva* and *Penicillium expansum*, have been found in canned foods and the latter can grow under vacuum also.

Carbonated beverages are usually not spoiled because of inhibitory effect of CO_2 on microorganisms. The acidity (resulting from carbonation and addition of acids) also inhibits microorganisms. Since molds require air, they do not grow on the carbonated beverages.

Spoilage of Jams, Jellies, Marmalades and Preserves

Jams and jellies have usually low water activity (0.75-0.86). In addition, due to added acid, the pH is lowered and sometimes may have preservatives such as benzoic acid. All these factors lower down the risk of spoilage. Also due to heating, spoilage causing organisms (yeasts, molds) are eliminated until the package is opened whereupon recontamination could be expected (Plate 1).

Usually, osmophilic yeasts such as *Torulopsis*, *Xeromyces* and many other have been reported to spoil jams, jellies and preserves.

Spoilage of Frozen Fruits

Many fruits and fruit products are preserved by freezing including cherries, fruit juice concentrates, purees (^{o}B>45) and some sliced fruits. Usually, dry sugar or syrup is added to fruit prior to freezing. The predominant microorganisms are usually yeasts and molds besides lactic acid bacteria (in orange juice concentrate).

Spoilage of Dried Fruits and Vegetables

In dried fruits (apples, apricots, dates, figs, peaches, prunes, resins etc.), a number of microorganisms can be expected. Due to decreased water activity (<0.65 in case of sun dried products), heat treatment during dehydration and fumigation, the microorganisms may be killed or unable to cause spoilage. But, spores of bacteria and molds are likely to be the most numerous. Dried fruits may be spoiled due to the development of rancidity as concentrated flavonoids may undergo oxidation. Dried or partially dried fruits (dates, figs and prunes) are also susceptible to yeast spoilage i.e. *Zygosaccharomyces*.

4.8 PREVENTIVE MEASURES

While the fresh fruits and vegetables are spoiled by biological (microbiological) and non-biological causes, their spoilage is checked by adopting suitable preservative techniques. But the processed products do not normally spoil unless the preservative technique applied is not proper or is not applied properly or the product is stored improperly. There are a few generalized preventive measures which can be adopted to avoid their spoilage.

- It is desirable to keep the initial microbial contamination as low as possible. The commodities should be handled and stored to avoid further contamination and create conditions to check the growth of microorganisms.
- All efforts be made to apply the preservative technique, keeping in view the various steps to avoid spoilage.
- Mechanical disruption of the processed product tissue should be prevented. Equipments used for handling should be clean and free from contamination and contamination from the soil microflora should be avoided. Dipping of fruits and vegetables in solution of chlorine (50-125 ppm) removes the adhering microflora.
- Inhibition of microbial growth can be achieved by storing the food at low temperature or in inert atmosphere packaging.
- While packaging the processed product, it is absolutely essential that the environment of packing should be microbes free or least contaminated and away from stores to minimize the post-processing contamination.
- The chemical and microbiological quality of water is the single most factor which can control the quality of the finished processed product. It should conform to the prescribed standards of microbiological (indicator microorganism) and chemical quality.

- The spoilage of canned products can be minimized especially leakage by regularly checking the equipments used in canning (reformers, flanger, double seamer, retort).
- The quality of raw material is controllable factor which have profound influence on the spoilage behaviour of processed product.

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 the food can get spoiled?

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2. List various causes of spoilage of food?

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3. Enumerate various principles used in preserving food?

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4. What are the major causes of spoilage of canned fruits and vegetable?

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5. Can dehydrated fruits and vegetables also get spoiled if so why?

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6. What is meant by acetification, putrefaction, rancidity, fermentation?

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7. What is meant by hard swell, Hydrgen swell and flat sour?

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8. What is meant by water activity?

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9. Can jams be spoiled?

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10. Name a few microorganisms involved in spoilage of juices.

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11. Can the carbonated juices also get spoiled? If so how?

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12. Enumerate various factors responsible for microbial spoilage.

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13. How would you classify the foods based on perishability?

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14. Classify the microorganisms according to their optimum temperature of growth?

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15. Name the bacterium responsible for causing flat sour.

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16. What is the relationship of initial number of microorganism with spoilage?

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17. Enlist five ways the spoilage can be prevented.

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4.9 LET US SUM UP

The foods especially fruits and vegetable are a living commodities and are therefore, liable for spoilage. The nature and kind of spoilage, however, depend upon the type of food and the environment where it is kept or how it is handled and stored. The causes of spoilage include contamination with microorganisms, activity of microorganisms or their enzymes, activity of native enzymes of food, infestation with rodents and influence of various external conditions where the food is stored. Decomposition of food constituents such as proteins, fats and carbohydrates and their interactions result into production of chemicals with different quality and hence, not acceptable as a food. To prevent spoilage, the foods are preserved either by giving low temperature during storage, or heated in appropriate media, irradiated, dehydrated or preserved with chemicals or made into fermented products. If such methods are not properly employed the foods can get spoiled even when they are processed.

4.10 KEY WORDS

Spoiled food : A food is said to be spoiled if it has been damaged or injured making it unsuitable for human use.

Perishable foods : There are the foods which spoil readily unless special preservation methods are used such as meat, fish, most of fruits and vegetables, egg, poultry and milk etc.

Non-perishable or stable foods : These foods do not spoil until and unless they are handled carelessly such as cereals, sugar etc.

Putrefaction : The anaerobic decomposition of the protein, peptide or amino acid resulting in the production of obnoxious odour is called as putrefaction.

Psychrophiles : Those microorganisms which have affinity for low temperatures (8-10^{o}C).

Mesophiles : Are the microorganisms which grow best at medium temperatures (25-40^{o}C).

Thermophiles : These microorganisms appear at higher temperatures (50-55^{o}C).

Under processing : It denotes insufficient or improper heat treatment that may result in survival of certain microorganisms causing spoilage of food product during their subsequent storage.

Water activity (aw) : Moisture content is usually expressed in terms of water activity.

Under-exhausting : If the air entrapped in the tissues of canned fruit and vegetables and the filling medium is not expelled properly, is called under-exhausting.

Panelling or buckling : It occurs in case of big sized cans when there is very high vacuum inside the can, atmospheric pressure can struck or force the can inwards resulting in leakage type of spoilage.

Flipper : A can with mild positive pressure is called a flipper.

Springer : A mild swell at one or both ends of a can is called a springer. One end may also remain permanently bulged and other flat.

Swells : In this case, both ends remain bulged. It may be a soft swell or hard swell.

Leaker : A can with a very small leak due to faulty seam, faulty lock seam or pinholes resulting from corrosion from inside of the can or rusting of the can from outside is called leaker.

Breather : It denotes tiny leak in the can that may allow air to pass back and forth into the can but not the microorganisms. The inside pressure equals outside pressure.

Buckled cans : Sometimes, the vacuum in big sized cans is so high that atmospheric pressure can strike the can body resulting in deformation of can which may further cause leakage of contents or contamination.

4.11 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

1. Your answer should include the following points:

   A food is a living commodity and respire and undergo various metabolic changes which if unchecked can spoil the food or if it is not harvested at proper maturity, is contaminated with dirt, or the microorganisms.

2. Your answer should include the following points:

   The major causes of spoilage are: the microorganisms or their enzymes, the native enzymes of food, rodents, environmental factors and purely chemical reactions.

3. Your answer should include the following points:

   - Delay or prevention of microbial decomposition of food.
   - Delay or prevention of self decomposition of food.
   - Prevention of damage to food caused by insects, rodents, birds and mechanical causes.

4. Your answer should include the following points:

   - Microbiological spoilage
   - Spoilage by physical causes

5. Your answer should include the following points:

   Dried fruits can have a number of microorganisms but due to decreased water activity, heat treatment during dehydration and fumigation, the microorganisms may be killed so unable to cause spoilage, but, spores of bacteria and molds may survive and cause spoilage.

6. Your answer should include the following points:

   Acetification: It is the process of conversion of ethanolic liquid into acetic acid by the activity of acetic acid bacteria.

   Putrefaction: The anaerobic decomposition of the protein, peptide or amino acid results in the production of obnoxious odour which is called as putrefaction.

   Fermentation: It is the process in which the organic compounds are converted into other organic compounds with generation of energy.

   Rancidity: It implies the oxidation of fatty substances giving specific off-odour.

7. Your answer should include the following points:

   In hard swells, both the ends of can remain bulged and rigid. In case of soft swell, the ends are not so hard. On applying some pressure, the ends may go inward (normal) but do not remain normal on the removal of pressure.

8. Your answer should include the following points:

   Water activity (aw): Moisture content is usually expressed in terms of water activity.

9. Your answer should include the following points:

   Jams and jellies have usually low water activity (0.75-0.86) so have lower risk of spoilage. However, if package is opened chances of recontamination could be expected or if there is leakage the product could be spoiled by osmophilic yeasts such as *Zygosaccharomyces Torulopsis*, *Xeromyces*.

10. Your answer should include the following points:

    Fungi

    Mucor,
    Rhizopus,
    Penicillium,
    Aspergillus,
    Alternaria, Cladosporium,
    Byssochlamys

Bacteria

Clostridium butyricum
B. coagulans (flat sour)
E. coli
Lactobacillus,
Leuconostoc mesenteroides
Streptococcus viscosum

Yeast

Saccharomyces

11. Your answer should include the following points:

    Carbonated beverages are usually not spoiled because of inhibitory effect of CO_2 on microorganisms. The acidity (resulting from carbonation and addition of acids) also inhibits microorganisms. Since molds require air, they do not grow on the carbonated beverages but may develop at the surface of uncarbonated soft drinks which contain air above the liquid surface.

12. Your answer should include the following points:

    Following are some of the factors responsible for spoilage: Number and kind of microorganisms, Suitability of temperature, Suitability of food, pH of food and Presence of air

13. Your answer should include the following points:

    Perishable foods: are the foods which spoil readily unless special preservation methods are used (meat, fish, most of fruits and vegetables, egg, poultry milk).

    Semi-perishable foods: Semi-perishable foods like waxed potatoes and some varieties of apple, if handled and stored properly, shall remain unspoiled for a fairly long period.

    Non-perishable foods: are the foods that do not get spoiled until and unless they are handled carelessly such as cereals, sugar.

14. Your answer should include the following points:

    Psychrophiles: These have affinity for low temperatures (8-10^{o}C).

    Mesophiles: These microorganisms grow best at medium temperatures (25-40^{o}C).

    Thermophiles: These microorganisms appear at higher temperatures (50-55^{o}C).

15. Your answer should include the following points:

    Bacillus coagulans

16. Your answer should include the following points:

    The initial number of microorganisms present on the food has a direct relationship with its spoilage. More the number of microorganisms present, rapid is the spoilage.

17. Your answer should include the following points:

    1) Keep the initial microbial contamination as low as possible and create conditions to check the growth of microorganisms.

    2) All efforts be made to apply the preservative technique.

    3) Mechanical disruption of the processed product tissue should not occur.

    4) Inhibit microbial growth by storing the food at low temperature or in inert atmosphere packaging of dried fruits and vegetable products.

    5) Equipments used for handling should be clean and free from contamination.

4.12 SOME USEFUL BOOKS

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UNIT 5 CONCEPT, DETERMINATION OF PROCESS LETHALITY REQUIREMENTS AND IMPORTANCE

Structure

5.0 OBJECTIVES

After studying this unit, you should be able to understand:

- kind of foods based on their acidic reaction;
- relationship between pH of foods and heat resistance of microorganisms;
- difference in heat resistance of vegetative cells and spores of microorganisms;
- what is thermal death time and how it is determined?;
- how microorganisms behave under freezing and refrigeration conditions?; and
- the basic principle involved in various methods for controlling microorganisms.

5.1 INTRODUCTION

The foods, which we eat or drink are also excellent substrates (food) for microorganisms, which are present in air, water, soil, utensils and even in raw foods. Under suitable conditions of growth, particularly temperature and moisture, the microorganisms multiply using these food items and produce luxuriant growth. Many foods serve as carrier of various pathogenic and non-pathogenic microorganisms, which may spoil the food by their growth, change of chemical nature of food, release of unpleasant odour, production of various harmful enzymes and toxins. Such foods are unfit for human consumption. For these reasons, it is essential to prevent the entry and growth of microorganisms in our food if present, by suitable processing. Before using a suitable process, we should understand various factors which may influence the effectiveness of a process.

5.2 CLASSIFICATION OF FOODS ACCORDING TO pH

Most foods are derived either from plants or from animals. In this course, we are concerned with foods of plant origin and are known as vegetables or fruits based on their use. These foods have different pH and are classified as low acid foods, medium acid foods, acid foods and high acid foods.

a) Low acid foods

The foods having pH above 5.3 are called low acid foods. For example: peas, corn, lima beans etc.

b) Medium acid foods

The foods which have pH between 4.3 and 5.3 are called medium acid foods. For example: asparagus, beets, pumpkin, spinach etc.

c) Acid foods

Foods which have pH between 3.7 and 4.5 are called acid foods. For example: pears, pineapple, tomatoes etc.

d) High acid foods

Foods having pH 3.7 or lower are included in this category. For example: Berries and sauerkraut.

You must have noted that in general vegetables are low or medium acid foods while fruits are acid or high acid foods.

Most foods are subjected to heat treatment or cooked before use. The heat process is essential in the canning of foods to eradicate the microorganisms, which may be present in the raw food or may enter from the environment during processing; and may spoil the food if not eradicated.

The effect of pH of the food is complicated as the heating at high temperature causes decrease in the pH of low or medium acid foods. Higher the original pH, the greater the drop of pH by heating. Foods artificially adjusted to more alkaline pH give increasing protection to spores against heat as pH increases towards 9.

5.3 RELATIONSHIP BETWEEN pH OF FOOD AND HEAT RESISTANCE OF MICROORGANISMS

The pH of the foods influence the heat resistance of microorganisms. In general cells or spores are most heat resistance in a substrate that is at near neutrality. An increase in acidity or alkalinity hastens killing by heat. However, a change towards acidic pH is more effective than a corresponding change in alkalinity. This will be more clear from the Table 5.1, which shows the effect of pH on heat resistance of spores of *Bacillus subtilis.* Therefore low acid foods are heated under pressure (i.e. temperature above 100^{o}C) while the high acid foods are heated up to 100^{o}C for making free from microorganisms.

Table 5.1: Effect of pH on heat resistance of spores of *Bacillus subtilis* in 1:15 M phosphate buffer (100^{0}C)

pH	Time of survival (min)
4.4	2
5.6	7
6.8	11
7.6	11
8.4	7

5.4 HEAT RESISTANCE OF MICROORGANISMS AND SPORES

The heat resistance of microorganisms varies widely within the species and their forms:

- Thermophiles are more resistance than mesophiles and psychrophiles are least resistance.
- Spores formers are more resistant than non-spore formers. Cocci are usually more resistant than rods.
- The bacteria that clump considerably or form capasules are more resistant to heat than those which do not.
- Cells high in lipid content are difficult to kill than cells having low lipid.

However, there are many notable exceptions to the above mentioned general statements.

Higher the optimal temperatures for growth, the greater the resistance to heat. Thermal death time of bacterial cells of a few microorganisms are exemplified in Table 5.2.

Table 5.2: Thermal death time of bacterial cells

Bacteria	Time (min)	Temperature (^oC)
Gonococcus	2 – 3	50
Salmonella typhosa	4.3	60
Staphylococcus aureus	18.8	60
Escherichia coli	20-30	57.3
Streptococcus thermophilus	15	70-75
Lactobacillus bulgaricus	30	71

The heat resistance of microbial spores is much higher than the vegetative cells, and vary with the species of microorganism and conditions during sporulation. Resistance may vary from <1 min to 20 h at 100^{o}C. Similar to non-spore forming species, the spore forming species which have higher optimal temperature for growth are more resistant to heat than those spore forming species having lower optimal growth temperatures.

Simultaneous growth of two spores formers enhances the resistance of spores having lower heat resistance, e.g. *Clostridium perferingens* growing with *C. sporogenes*. Thermal death times of spores of a few microbial species are given in Table 5.3.

Table 5.3: Thermal death times of bacterial species

Species	Thermal death at 100^{o}C (min)
Bacillus anthracis	1.7
Bacillus subtilis	15-20
Clostridium botulinum	100-300
Clostridium calidotolerans	520
Bacillus coagulans (flat sour bacteria)	> 1030

Above examples of thermal death times of vegetative cells as well as of spores are at various concentrations of cells or spores in different substrates. These values may change to lower or higher under different conditions.

What happens to enzymes in food by heat treatments?

Most foods and microbial enzymes are destroyed at 79.4^{o}C, however some may withstand higher temperatures, especially if high temperature for short duration is employed. This is called **pasteurization**, which you will learn later in this course.

5.5 THERMAL DEATH POINT (TDP)

Thermal death point is the lowest temperature at which all microorganism in a liquid suspension are killed in 10 minutes.

5.6 THERMAL DEATH TIME (TDT OR t_D)

The thermal death time is defined as the time required, at a given temperature, for heat killing of a population of a single species of microorganism in aqueous suspension. t_D depends on the size of the population and on the pH of the suspension. It is an important factor for controlling the microorganisms by heat treatment or to determine the heat resistance of a microorganism.

5.7 DETERMINATION OF THERMAL DEATH TIME (t_D)

The description of all the procedure and equipments/apparatus, used in the determination of thermal death time is beyond the scope of this course. However, a simple glass-tube method, used in canning industry is discussed here.

5.7.1 Glass Tube Methods

A known population of cells of an axenic culture in a small volume (1 ml) of buffer solution is sealed in small glass tube. The tubes are heated in a thermostatically controlled bath to a selected temperature. The tubes are selected periodically, cooled immediately to 0^oC and the population of viable cells is determined. In case of spores, the suspension is first pasteurized to kill the vegetative cells, if present, before subjecting the spore suspension to d_T test. This is necessary as the lysed vegetative cells may have protective effect on spore-population.

Care is also taken to break up the clumps and remove the growth medium by centrifuging and washing. The volume of microbial suspension added to the buffer is kept 1-2 percent to avoid the change in the composition of heating substrate and the vials containing the suspension are brought to constant temperature, usually 0^oC before subjecting to heat treatment. If temperature above 100^oC is selected, oil bath instead of water bath is used. The test is always made in multiple tubes. Viability of the surviving organism after heat treatment should be checked on appropriate medium containing all the nutrients, which support maximum growth of that organism.

5.7.2 Decimal Reduction Time

When a microbial population is heated, the cells die at a constant rate. For example, suppose a population of 1 million (10^6) cells has been heated to a high temperature for 1 minute and 90% has died. We are now left with 100,000 (10^5) cells. If the leftover population is heated for another 1 minute, 90% of the population leaving 10000 (10^4) survivers. Thus the each one minute of heat treatment will reduce 90% of the remaining population. This is shown in Table 5.4 and is known as decimal reduction time (DRT) and represented by *D*. It can be defined as the time of heating at a temperature to cause 90% reduction in the population of viable cells or spores.

Table 5.4: Microbial death rate at constant temperature

Time (min)	Death min^{-1}	Number of survivors
0	0	1,000,000
1	900,000	100,000
2	90,000	10,000
3	9,000	1,000
4	900	100
5	90	10
6	9	1

The D value (Decimal reduction time) may also be defined as the 'time at given temperature for the surviving population' to be reduced by 1 log cycl. Please refer Figure 5.1, if we extrapolate the times from 10^3 and 10^2, the time difference is (3.5 – 2.5 = 1min is D). It means within 1 min initial population will decrease by 90 per cent (from 1000 to 100, Difference 1000 – 100 = 900).

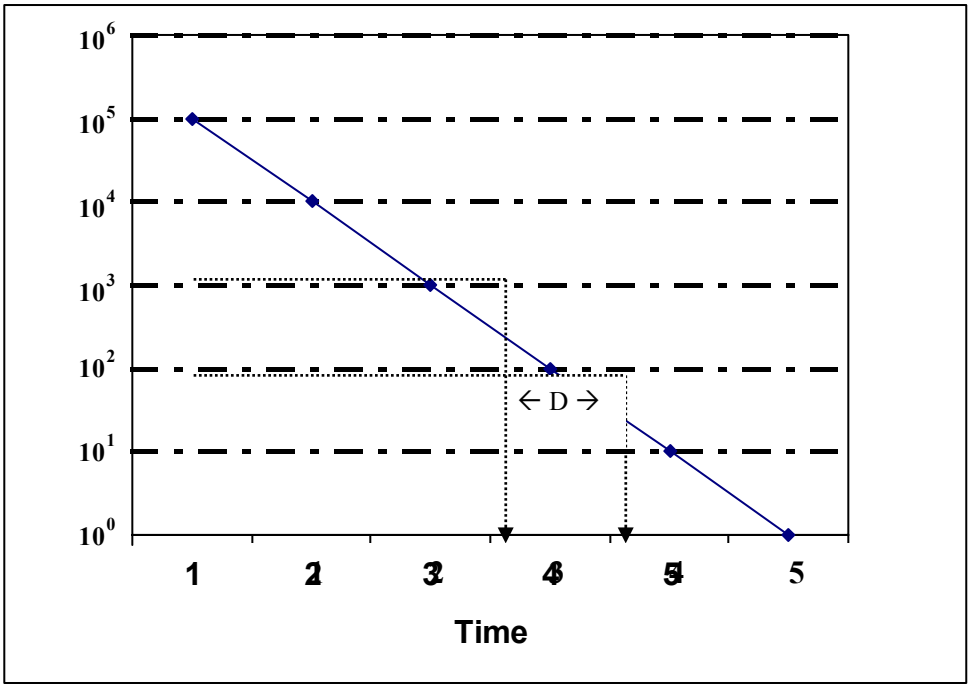

Figure 5.1: A microbial death curve showing constant death rate of cells i.e. 90% per minute D value may be calculated from the curve by extrapolating lines from the Y axis and calculating the time difference.

5.7.3 Thermal Death Time Curve (TDT Curve)/Kinetics

The methods to construct TDT curve are: (i) The growth – no growth method (ii) classical end point method and (iii) based on *D* values. We shall here discuss the method based on *D*-values. D values can be calculated at different temperatures (refer 5.7.2). As the temperature is increased, the D value decreases. It means if we heat the sample at high temperature, it will take less time to kill the microorganism in a given food sample. If we plot log D values against temperature, we will get a straight line. From this we can derive

another important parameter in heat processing Z, the temperature change which results in a ten fold (1 log) change in D.

$$Z = (T2 - T1)$$

D value for a known population of cells or spores of a microbial species at several temperatures can be estimated. By plotting D values on the logarithmic scale against temperature TDT curves can be constructed (Figure 5.2).

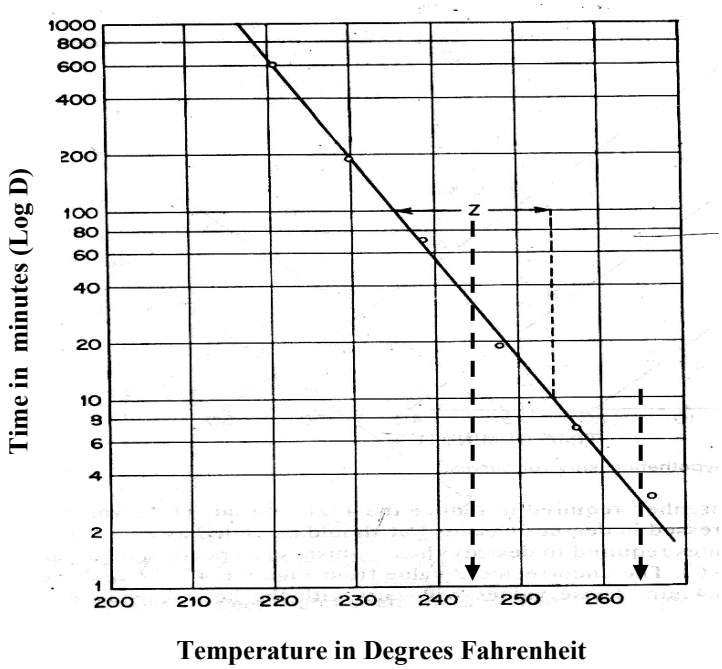

Figure 5.2: TDT curve for spores of flat sour bacteria; 115,000 spores per ml in corn at pH 6.1 (z = 19).

If we are interested to process the food item so that it may be free from any spore or microorganisms, first we have to calculate D, Z and F values. *F* is the time in minutes required to destroy the organism in a specified medium at 121°C. These values vary with the heat resistance and concentration of the test organisms and with the medium in which it is heated. From the *Z* and *F* values process times can be calculated.

5.7.4 12D Concept

Canned foods are susceptible to the spores of the organism *Clostridium botulinum*, this organism causes botulism. As a safety measure, the canning industry use the 12*D* heat treatment for low acid foods. In this process enough heat is provided to reduce 10^{12} spores of *C. botulinum* to 1 spores per ml. It can be explained as follows.

Assuming that *D* has a value of 0.21 minutes for spores of *C. botulinum* at 121°C and that out of 12 cans of food contains 1 spore. A heat process at 121°C for 2.52 min would reduce the spores to 1 spore in 10^{12} cans. The value of 2.52 min has been arrived by the following formula:

$$F_o = D_{121} (\log a - \log b)$$
$$= 0.21 (\log 1 - \log 10^{-12})$$
$$= 0.21 \times 12$$
$$= 2.52$$

5.8 DETERMINATION OF PROCESS LETHALITY REQUIREMENTS AT LOW AND HIGH TEMPERATURE

5.8.1 Heat Penetration

In heat/thermal processing of foods, the ratio of penetration of heat into a food is very important, because every part of food in a container must get adequate heat to prevent spoilage. The part which heats most slowly is the critical one; and this is near the centre of container. Solids are heated by conduction while liquids by convection. Foods which are semisolid are heated by combination of both conduction as well as convection. Conduction is slow in foods and rapid in metals.

The factor that determine the time required to bring the centre of the container of food upto the sterilizing temperature are as follows:

i) Material of which the container is made

Glass has slower rate of penetration than metal (tin can).

ii) Size and shape of container

Large container takes long time than small container. Less the radius of container faster the heating. For example: long slim cylinder will heat faster than compact wide cylinder of the same volume.

iii) Initial temperature of food

For the foods with the higher initial temperature the average temperature during heating is higher than in foods having lower initial temperature. A high initial temperature is important in processing canned foods that heat slowly. For example: pumpkin, cream style corn etc.

iv) Initial temperature of retort (steam sterilizer)

Fastest heating takes place in initially hot retort than retort having initially low temperature. Therefore, preheated retort should be used.

v) Consistency of food, size and shape

Consistency of food, size of food pieces and even their shapes affect the penetration of heat. The changes that takes place during heating (cooking effect) also affect the heat penetration. Penetration of heat in large pieces takes more time than in small pieces. This is applicable in foods, which retain their shape and even size during heating. For example: peas, plum, beats, whole grain corn etc.

Some foods become mushy or viscous during heating. In such foods penetration is slow. For example: Sweet potatoes, pumpkin, etc.

Pieces that layer like asparagus layers or spinach layers interfere with convection current

Sauces added on baked beans slow down heat penetration more than plain sauce. Starch interferes increasingly as the concentration is raised. Sodium chloride is never added in high concentration as it slows down rate of heating. Rate of heat penetration also decreases with increasing

concentration of sugars; however this effect is counteracted some what by the marked decrease in the viscosity of sugar solutions. *(Addition of sugar and salt slow down the rate of heating)*

vi) Rotation and agitation

The rotation and agitation of the container of food during heat processing hasten heat penetration, if food is in form of fluid. However, in some food such operation may cause undesirable physical changes.

5.8.2 Cooling of Food after Heat Processing

The cooling operation involves the same principles of heat transfer as the heating process. Rapid artificial cooling is recommended as slow cooling may cause overcooking of the food and may allow the growth of thermophiles.

5.8.3 Determination of Thermal Processes

To determine the thermal processes data on the following two aspects are required:

i) TDT curve for most heat-resistant organism likely to be present in food. For example in low acid foods spores of *Bacillus coagulanse* (flat sour organism), which is a thermophile, may be present.

ii) Heat penetration and cooling curves for the food when packed in specific type of container of fixed size.

There are three methods to determine thermal processes:

- Graphical methods
- Formula method
- Nomogram method

The principle is similar for all the three methods; however the graphical method is most simple and therefore explained here:

Graphical method to determine thermal processes

1. The TDT curve for the most heat resistance organism likely to be encountered is determined in food being canned.

   TDTs from this curve are converted to **lethal rates** for the various heating temperatures. The lethal rate for a temperature is the reciprocal of the TDT. If TDT is 400 min at 126.7^{o}C to kill the spores in a food, the lethal rate would be 1/400 i.e. 0.0025.

2. Heat penetration and cooling curve for the food and can size involved are determined.

3. The lethal rates for different temperatures at the centre of can during the length of heating and cooling process are plotted on the heat penetration or cooling curves (see Fig. 5.3). In this figure the lethal rates are 0.01 units and times are 10 minutes for a square. An area equivalent to 10 squares under the lethality curve is unity. This means that the destruction of all the spores or cells has been accomplished. If this area is less than unity (i.e. less than 10 squares), the process is inadequate, and if more than 10 squares, it is greater than needed. The area beneath the curve is

measured by planimeter. In Figure 5.3 the heat treatment of 56 min at 126.7°C and 78 min at 121°C are adequate.

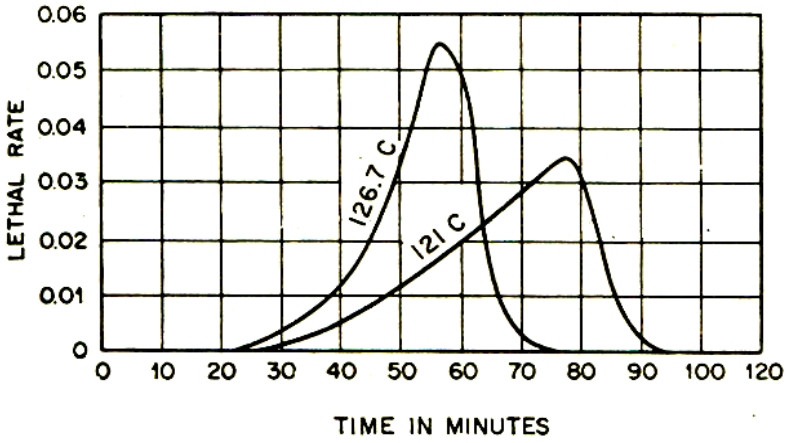

Figure 5.3: Equivalent lethality curves with retort at 126.7°C. (50,000 spores per ml)

5.9 BEHAVIOUR OF MICROORGANISMS UNDER FREEZING AND REFRIGERATION ENVIRONMENTS

Low temperatures are used to retard chemical reactions, and actions of food enzymes and to reduce slow down or stop growth and activity of microorganisms in food. The lower the temperature, the slower will be the chemical reactions, enzyme and microbial growth; and temperature below certain level will prevent the growth of a microorganism. Each microorganism has a specific **Cardinal temperature** i.e. the minimal temperature and maximal temperature at which it can grow and optimal temperature at which the growth is fastest in the shortest time. As the temperature drops below the optimal temperature towards the minimal, the rate of growth of organism decreases and is slowest at the minimal temperature. Below minimal temperature, the growth will stop but slow metabolic activity may continue. Therefore cooling down of a food from normal temperature has different effects on various microorganisms and slow down the growth of others; however the extent would vary with different species and even strains of microorganisms. A further decrease of 10°C would stop growth of more organisms, and make still slower the growth of the others. Therefore storage at low temperature influence the type of spoilage microorganisms which may predominate as illustrated in Table 5.5.

Table 5.5: Growth rate of Pseudomonas fragii at various temperatures

Temperature (°C)	Exponential growth rate (generation h^{-1})
0.0	0.09
2.5	0.13
5.0	0.20
7.5	0.29
10.0	0.38
20.0	0.92

5.9.1 Growth of Microorganisms at Low Temperature

In general, freezing prevent the growth of most food born microorganisms and refrigeration slow down growth rates except for *Clostridium botulinum* type E. Temperature below 5-6^{o}C or less, effectively retard the growth of most food spoilage microorganisms. However, some of the microorganisms may survive at subfreezing temperature in frozen food. (Table 5.6 and 5.7)

Table 5.6: Microorganisms able to grow at subfreezing temperatures

Organisms	Temperature (^{o}C)
Molds	
Claedosporium	–6.7
Sporotrichum	–6.7
Penicellium	–4.0
Monilia	–4.0
Yeast	
Yeast (one strain)	–34.0
Yeast (two strains)	–18.0
Bacteria	–5.0 to –17.8

Table 5.7: Different microorganisms able to grow in different frozen foods

Organisms	Food	Temperature (^{o}C)
Bacteria	Meat	– 5.0
	Cured meats	–10.0
	Fish	–11.0
	Vegetables	–12.2
	Ice cream	–10.0
Yeast	Meat	– 5.0
	Oysters	–17.8
Molds	Meat	– 7.8
	Vegetables	– 7.8
	Barries	– 6.7

5.9.2 Effect of Freezing and Subfreezing Temperature on Microorganisms

Feezing usually results in a considerable reduction in the number of viable organisms in a food. The reduction in recoverable numbers can be the result of lethal or sublethal effects.

a) Lethal effects

Though several cells of microorganisms are killed by freezing, a few may remain viable with little or suspended metabolic activity. The lethal effects

are due to denaturation or flocculation of essential cell proteins or enzymes possibly as a result of the increased concentration of solutes in the unfrozen water or perhaps in part because of physical damage by ice crystal. Rapid cooling of cells from an optimal temperature to $0^{o}C$ is most injurious and may lead to cell death due to cold shock. It is probably due to crystallization of the liquids in the membrane which damage to the permeability of the cell or due to the release of repair enzyme inhibitors, e.g. **ribonuclease inhibitors.**

b) Sublethal effects

Freezing of food may cause cryo injury to the microorganisms present on food. Such injured cells are referred as freeze injured, frost injured, cryo-injured or metabolically injured. Such cells are not really dead and may recover to start refunctioning, if repair time is permitted or additional nutritional factors are added to the enumeration media. This fact of cryoinjury is of great significance in the microbiological examination of foods.

5.9.3 Factor Affecting Microorganisms during Freezing

1. The resistance or sensitivity of microorganisms vary with the kinds of microorganisms, their form and growth phase. For example:
   a) Thermophiles are most sensitive and psychrophiles most resistance.
   b) Spore formers are more resistance than non-spore formers.
   c) Bacteria in logarithmic phase are more sensitive than in stationary phase.
   d) Rods are more sensitive than cocci.
2. Microorganisms are classified on the basis of sensitivity to freezing:
   i) **Susceptible:** Gram negative bacteria and vegetative cells of yeast and molds.
   ii) **Moderately resistant:** Gram positive streptococci and enterococci.
   iii) **Insensitive:** Spore formers.
3. Freezing parameters:
   i) ***Freezing rate***

      Rapid cooling upto $0^{o}C$ is injurious to microbial cells.

   ii) ***Freezing temperature***

      Freezing temperature between –4 to –10 is more injurious than –15 to $-30^{o}C$.

   iii) ***Times of frozen storage***

      Maximum death of microorganisms occur during the freezing process. Once the temperature is stabilized, the death is less and very low.

   iv) ***Kinds of food***

      The composition of food influence the rate of killing of microorganisms during freezing and storage. Sugar, salt, protein,

colloids, fats and other substance in foods provide protection to the microorganisms while low pH and high moisture hasten the death.

v) ***Slow defrosting of food cause death*** of microorganisms.

vi) ***Freezing and thawing***

Maximum casualty of microorganisms occur if foods are repeatedly frozen and thawed.

5.9.4 Effect of Freezing on Constituents of Microbial Cells

With the lowering of temperature, water in cell gets frozen. As a result the unfrozen fluids in cell gets concentrated with solutes (salts, proteins, nucleic acids, etc). This may change the pH of cellular matter, concentrate electrolytes, alter colloidal states, denature proteins and increase viscosity. Ice crystals also form outside the cell due to the freezing of water molecules in food. These extracellular ice crystals draw water from the cell causing dehydration or concentration effect. Intracellular ice crystals due to the freezing of water may rupture cell membrane and alter the permeability of cell membrane. Intracellular ice crystals are more injurious than the extracellular ice crystals to the microbial cells.

5.10 CONTROL OF MICROORGANISMS BY VARIOUS MEANS

Most foods are either of plants or animal origin. Their spoilage is prevented by controlling microbial growth on them by using various methods. Some important methods are listed below:

1. Asepsis or preventing contacts with microorganisms.
2. Killing the microorganisms by heat treatment.
3. Keeping away from the microorganism e.g. maintenance of anaerobic conditions in sealed or evacuated container.
4. Storage at low or ultra low temperature
5. Drying.
6. Increasing osmotic concentration in foods.
7. Mixing with preservative.
8. Change of pH
9. Mechanical destruction in industry: grinding or high pressure.

Usually a combination of more than one method is used to control the microbial spoilage of food. For example: canned foods are preserved by heat treatment followed by evacuation and sealing of can. Similarly, many processed foods involve heating, mixing with preservative, evacuation of air and sealing.

5.11 PRINCIPLES INVOLVED IN VARIOUS METHODS TO CONTROL MICROBIAL SPOILAGE OF FOOD

The basic principle involved in various methods to control microbial spoilage of foods are prevention or delay of microbial decomposition. These are achieved by followings methods:

a) By keeping out microorganisms. For example: Aseptic condition during processing of food.

b) By the removal of microorganisms: For example

- By removing the microbially infected portion of food, covering, skin etc.
- Washing of raw food.
- Filteration.

c) By hindering the growth and activity of microorganisms by low temperature, drying, anaerobic condition or chemical.

d) By killing the microorganisms: sterilization by heat or radiation.

5.12 LET US SUM UP

Human foods are excellent substrate for various microorganisms, which are present in air, water, soil, raw foods, on the body of living and non-living organisms. These microorganisms may harm the body if enter in the body/tissue due to rupture or injury of outer covering layer. Many of these microorganisms may enter the food during the processing and remain either dormant for long time till get the right conditions for multiplication, if consumed, cause unpleasant odour or taste making the food unfit for consumption. Such spoilage is called microbial spoilage of food.

Prevention of microbial spoilage of foods depend upon the kind of foods. The acidity or pH of the food influence heat resistance of microorganisms. Microbial cells are more resistance to heat at pH near to neutrality and therefore, low acid foods are heated under pressure to kill the microorganisms, where as the high acid foods are heated upto 100^{o}C for short duration to make free from organisms.

The thermal death time (TDT) which is defined as the time required to kill a known population of microorganisms at a certain temperature by heat. This value differ for different microorganisms and also on various environmental factors. TDT values are of great significance in heat control of microorganisms.

The death of microbial cells is at constant rate and is expressed in *D*-value i.e. decimal reduction time. *D*-values of a microbial species at several temperatures are estimated and plotted against temperature to construct curve, which are used to determine the actual time required for killing microbes in a food at a particular temperature. In practice 12*D* concept is used in canning industry. This is based on the reduction of 10^{12} spores ml^{-1} of *Clostridium botulinum* in low acid food to 1 spore ml^{-1} by heat treatment.

Low temperature under freezing and refrigeration reduce microbial growth and at 0^{o}C most of the microorganisms stop the growth. However a few exceptions of molds, yeast and bacteria, which may grow up to −6 to -38^{o}C have been encountered.

Freezing may have lethal or sub-lethal effect on microbial cell. In sub-lethal effect the freezing injury makes the microbial cell unable to multiply, however under favourable conditions, such cells may get repaired and grow.

The various method for controlling microorganisms are asepsis, heat treatment, anaerobic conditions, storage at low temperature, drying, increasing osmotic concentration, mixing with preservatives, change of pH, irradiation and mechanical destruction by grinding or high pressure. The basic principle involving in these methods are; prevention or delay of microbial decomposition by keeping out or removing the microorganisms or creating suboptimal conditions for survival and growth of microorganisms or by killing the microorganisms.

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 acid foods?

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2. Based on acidic reaction of food, which foods require more heating time to kill the microorganisms?

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3. Which of the two is more resistant to heat and why? *Escherichia coli* or *Clostridium botulinum.*

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4. Define thermal death time.

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5. Define thermal death point.

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6. What do you understand by decimal reduction time?

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7. Why TDT curves are constructed?

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8. What is 12D concept?

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9. Thermal processing of food is affected by the ……. of food.

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10. Which method is most common and simple to determine thermal processes?

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11. Will all microorganisms stop growing, if the food is stored at 0°C?

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12. What is cryoinjury?

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13. Should the food be cooled rapidly to 0^{o}C to kill microbial cells?

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14. Intracellular ice cause …….

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15. Microorganisms in food can be controlled by ……...

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16. Spores are more resistant to heat than ……

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17. Which of the following microorganisms is insensitive to freezing:

    i) Escherichia coli
    ii) Vibrio cholerae
    iii) Clostridium botulinum

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    …….

18. Freezing of food is one of the important method to prevent their spoilage on what principle it is based?

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19. Most microorganisms should be killed but the enzyme should not be destroyed. What method of heat processing should be applied?

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20. If heat treated food is cooled slowly, what kind of microorganisms during storage may grow.

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    …….

5.13 KEY WORDS

TDP : Thermal death point is the lowest temperature at which all microorganisms in a liquid suspension are killed in 10 minutes.

TDT : Thermal death time is defined as the time required, at a given temperature, for heat killing of a population of a single species of microorganism in aqueous suspension.

D Value : Decimal reduction time is defined as the time of heating at a temperature to cause 90% reduction in the population of viable cells or spores.

12D Concept : A process in which enough heat is provided to reduce 10^{12} spores of *clostridium botulinum* to 1 spores per ml in canned food.

5.14 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   Food which have pH between 3.7 and 4.5. Example: pear, pineapple and tomatoes.

2. Your answer should include the following points:

   Low acid foods.

3. Your answer should include the following points:

   Clostridium botulinum as it is spore former.

4. Your answer should include the following points:

   Thermal death time is the minimum time required to kill a population of microorganisms in liquid suspension at certain temperature.

5. Your answer should include the following points:

   Thermal death point is the minimum temperature required to kill population of microorganimsm in liquid in 10 min.

6. Your answer should include the following points:

   By any killing agent, the death of microorganisms is at a constant rate. Suppose a population of 10^6 cells are subjected to heat treatment, 90% of them will die in first minute. Of the remaining population, 90% will die in next one minute and so on until all the population die.

7. Your answer should include the following points:

   TDT curves are constructed to determine the thermal process time at different temperature to kill the microorganisms in a food.

8. Your answer should include the following points:

   It is a concept of time required to reduce 10^{12} spores of *Clostridium botulinum* in 1 ml suspension to 1 spore by heating to certain temperature. It is used in canning industry.

9. Your answer should include the following points:

   Thermal processing is affected by the **consistency** of food.

10. Your answer should include the following points:

    Graphic method.

11. Your answer should include the following points:

    No; psychrophiles will continue growing.

12. Your answer should include the following points:

    Injury to microbial cells during freezing.

13. Your answer should include the following points:

    Yes.

14. Your answer should include the following points:

    Rupture of cell membrane leading to the leakage of cell constituent.

15. Your answer should include the following points:

    Sterilization

16. Your answer should include the following points:
    Vegetative cells

17. Your answer should include the following points:
    Clostridium botulinum

18. Your answer should include the following points:
    Reducing or suspending the metabolic activity and growth.

19. Your answer should include the following points:
    Pasteurization

20. Your answer should include the following points:
    Thermophiles

5.15 SOME USEFUL BOOKS

1. Adams, M.R. and Moss, M.O. (1995) Food Microbiology. Low cost Indian addition published by New Agro International (P) Limited Publishers, New Delhi 398p.
2. Frazier W.C. and Westhofl D.C. (1967) Food Microbiology. Tata McGraw Hill Publishing Co., New Delhi 540p.
3. Jay, J.M. (1970) Modern Food Microbiology. Van Nostrand Reinhold Co., London. 328p.

UNIT 6 THERMAL CONTROL OF MICROORGANISMS

Structure

6.0 OBJECTIVES

After studying this unit, you should be able to:

- make you understand the principals of food preservation using high temperatures;
- explain various processes for thermal preservation;
- describe the terms associated with heat preservation; and
- discuss the causes of spoilage of heat processed products and common spoilage organisms.

6.1 INTRODUCTION

The minute living organisms, not visible to the naked eye and classified as microorganisms, are virtually everywhere. Those of primary medical interest are bacteria, viruses, spirochetes, rickettsia, molds, and yeasts. They flourish in the soil of the farms that grow our grains, fruits and vegetables, on the hides and feathers of our meat animals and on the fins and organs of the seafood we eat. Though there are innumerable genera and species of each class of microorganisms, not all are of medical significance or involved in disease processes. Many of these organisms can be beneficial. In fact the predominance are composed of those that are necessary to food production, friendly environments, and metabolic processes, examples being cheese/wine

production, decomposition of organic matter, and digestion of food. Lactic acid bacteria in the dairy industry, yeasts in the baking and brewing industries, molds for specialty cheeses are examples of "domesticated" microorganisms. But in a many cases these microscopic flora create serious problems in our food supply. These problems fit into two categories. **Food spoilage** occurs when the food becomes unpalatable as the result of microbial growth. Products develop undesirable flavors, odors, appearances or textures via microbial action. The other, more dangerous problem is **food poisoning**, which occurs when the organisms present in food cause human illness or death. The microorganisms either produce a toxin or cause an infection, generally intestinal, when consumed. Those organisms that spoil product are typically called **spoilage organisms**, while those that can make people sick are referred to as **pathogens**. Therefore, to avoid both of these problems we need to understand the techniques which prevent their growth.

Food preservation has been around for a long time. The technique of food preservation may vary but the goal of food preservation has been the same **i.e. to keep the food in a stable condition over a period of time so that it will not spoil or make people sick.** There are various ways of food preservation, including chemical preservation, modified atmospheres, irradiation, low temperature preservation, preservation by drying and high temperature preservation.

6.2 THERMAL PRESERVATION OF FOODS

The most common method of killing microorganisms is to subject them to a heat treatment. High temperatures act by killing vegetative cells and also spores and denaturing the food enzymes. It may also act to destroy toxins produced by certain microorganisms.

The heat treatment used depends on the following factors. In order to safely preserve foods using heat treatment, the following must be known:

- What time-temperature combination is required to inactivate the most heat resistant pathogens and spoilage organisms in one particular food? The higher the temperature, the less time needed and vice versa. Heat destruction of microorganisms is a gradual phenomenon the longer is the treatment time at lethal temperatures, the larger is the number of microorganisms killed. As higher is treatment temperature the shorter is the time required to kill microorganisms and lower is heat induced damage to food products.

- What are the heat penetration characteristics in one particular food, including the can or container of choice if it is packaged?

- What are the types of micro-organisms present in the food material? The thermal death time of different microorganisms vary widely with the species. Different foods will support growth of different pathogens and different spoilage organisms so the target will vary depending upon the food to be heated.

- What is the concentration of the microorganisms? The higher the concentration, the more time is needed.

- What is the state of the microorganism? Spores are more resistant than vegetative cells. Organisms that have been stressed are more susceptible to heat.

- What is effect of heat on the product? Obviously, the temperatures required to kill microorganisms affect most food products.

- The degree of heat penetration also must be considered. Preservation processes must provide the heat treatment which will ensure that the remotest particle of food in a batch or within a container will reach a sufficient temperature, for a sufficient time, to inactivate both the most resistant pathogen and the most resistant spoilage organisms if it is to achieve sterility or "commercial sterility", and to inactivate the most heat resistant pathogen if pasteurization for public health purposes is the goal

- What is the effect of various environmental factors, such as pH and salts or solutes. Food acidity / pH value has a tremendous impact on the target in heat preservation/ processing.

Check Your Progress Exercise 1

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

1. Differentiate between spoilage organisms and pathogens?

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2. What is food preservation?

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3. Write various ways of food preservation?

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4. List important factors which need consideration in order to safely preserve foods using heat treatment?

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6.3 HEAT PRESERVATION PROCESSES

The most common type of food preservation by high temperatures is cooking. However, there are many more processes that involve the use of temperature above that of ambient air.

6.3.1 Sterilisation

By sterilisation we mean complete destruction of micro-organisms. Because of the resistance of certain bacterial spores to heat, this frequently means a treatment of at least 121° C (250° F) of wet heat for 15 minutes or its equivalent. It also means that every particle of the food must receive this heat treatment. If a can of food is to be sterilized, then keeping it at 121° C or retort for the 15 minutes will not be sufficient because of relatively slow rate of heat transfer through the food in the can to the most distant point. In such cases time needs to be increased.

6.3.2 Commercially Sterile Food Products

Sterile means free of life of every kind and is actually achieved under very limited conditions. The control of microorganisms in medicine, industry, sanitation, food, and feed service involves the acceptance that sterilization is most often not achievable without destroying or severely damaging the product. Only Low Acid Foods [LAF], having pH higher than 4.6, must be sterilized, because all microorganisms are able to grow in LAF. More acid products [pH equal/lower than 4.6] do not allow the growth of pathogenic spore forming bacteria. Then Sterilization is not required. Hence **Commercial Sterility** is a term commonly used in the canning industry meaning the condition achieved by the application of heat sufficient to render the processed product free from viable microorganisms (including those of known public health significance), capable of growing in the food under normal non-refrigerated temperatures at which the food is likely to be held during distribution and storage.

The process was developed by Nicolas Appert and published in 1810. All vegetative organisms that could grow in the food and cause spoilage under normal handling and storage conditions are destroyed. However **commercial sterile** foods may contain a small number of heat resistant bacterial spores, but they will not multiply under normal handling and storage conditions. Types of commercially sterile processes include canning, bottling, and aseptic processing. Commercial sterilization must make sure the numbers of surviving spores are at an acceptable level. The acceptable number of spores will depend on what type of damage they are capable of causing if they start to grow.

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 sterilization?

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2. What is commercial sterility? How commercial sterilization is different from sterilization?

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6.4 PASTEURIZATION

In the previous section you have read about strerilzation and commercially sterile foods. Now we will discuss milder heat treatment i.e. pasteurization. It is one type of preservation by heat that most people are familiar with. It is process of heating a liquid, particularly milk, to a temperature between 55 and 70 degrees C (131 and 158 degrees F), to destroy harmful bacteria. This process is named after the French chemist **Louis Pasteur**, who devised it in 1865 to inhibit fermentation of wine. Pasteur's aim was to destroy bacteria, molds, spores etc. He discovered that the destruction of bacteria can be performed by exposing them to certain minimum temperature for certain minimum time and the higher the temperature the shorter the exposure time required. Through this process, all of the bacteria (such as *E.coli, Lysteria*, and *Salmonella)* are not destroyed, it still exists in pasteurized products, but in very low concentrates. Refrigeration keeps the bacteria from further growth, very low. There are other bacteria that aren't harmful to humans, but they produce acids that turn the milk sour. They are called lactophilic because they consume the lactose in milk and produce acids. The extent of the pasteurization treatment required is determined by the heat resistance of the most heat-resistant enzyme or microorganism in the food. For example, milk pasteurization is based on *Mycobacterium tuberculosis* and *Coxiella burnetii*. These two organisms are the most heat resistant of pathogens that are not spore forming. Milk is a product that most people know is pasteurized. It is pasteurized by heating at a temperature of 63 degrees C (145 degrees F) for 30 minutes, rapidly cooling it, and then storing it at a temperature below 10 degrees C (50 degrees F).

Pasteurization is a comparatively low order of heat treatment, generally at a temperature below the boiling point of water. The more general objective of pasteurization is to extend product shelf-life from a microbial and enzymatic point of view. Pasteurization is frequently combined with another means of preservation - concentration, chemical, acidification, etc. Blanching is a type of pasteurization usually applied to vegetables mainly to inactivate natural food enzymes. Depending on its severity, blanching will also destroy some microorganisms.

Depending upon time and temperature treatment there are three kinds of pasteurization processes.

6.4.1 Low Temperature Long Time (LTLT)

Where pasteurization time is in the order of minutes and related to the temperature used; two typical temperature/time combinations are as following: 63°C to 65°C for 30 minutes or 75° C over 8 to 10 minutes. Pasteurization temperature and time will vary according to:

- nature of product; initial degree of contamination;
- pasteurized product storage conditions and shelf life required.

In LTLT pasteurization it is possible to define three phases:

- heating to a fixed temperature;
- maintaining this temperature over the established time period (= pasteurization time);
- cooling the pasteurized products: natural (slow) or forced cooling.

This is a typical batch method where a quantity of milk is placed in an open vat and heated to 63°C and held at that temperature for 30 min. Sometimes filled and sealed bottles of milk are heat-treated in shallow vats by that method and subsequently cooled by running water.

6.4.2 High Temperature Short Time (HTST)

HTST pasteurization is characterized by a pasteurization time in the order of seconds and temperatures of about 85° to 90° C or more, depending on holding time. Typical temperature/time combinations are as follows:

- 88° C for 1 minute
- 100° C for 12 seconds
- 121°C for 2 seconds.

While bacterial destruction is very nearly equivalent in low and in high pasteurization processes, the 121°C/2 seconds treatment give the best quality products in respect of flavour and vitamin retention. This is the most widely used process. The “hold time” is typically 125°C to pasteurize milk. This process is a continuous method and a “hold tube” is used. The “hold tube” is the tubing in the system that transports the milk after the point where the product is heated. The tubing is sized so that it takes 15-20 seconds for the product to travel all the way through it. When it reaches the end, if the temperature is at 125°C or hotter, it is considered pasteurized. It is then cooled and put in storage. The warm milk passes through the cooling section where it is cooled to 4° C or below by coolant on the opposite sides of the thin, stainless steel plates. The cold, pasteurized milk passes on to a storage tank filler for packaging.

6.4.3 Ultra High Temperature (UHT) Processing Treatments

In this method, milk is exposed to a brief, intense heating, normally to temperatures in the range 135-140 °C but for a very short time, a second or less. The treatment kills all microorganisms that would otherwise spoil the product. The process depends upon a fairly complicated *sterilizer/aseptic filling* design. The two stages of effective heat sterilization followed by aseptic filling represent an integral system. Frequently the packaging material for UHT milk is cardboard which must be chemically sterilized prior to the filling operation

This method is used mainly for coffee creamers and boxed juices with the exception of Europe. They pasteurize milk in this way. After this is done, there is no need to refrigerate, because it sterilizes the product. Sometimes the products can have a "cooked" taste that can be detected after being brought to such a high temperature.

Industrial applications of pasteurization process are mainly used as a means of preservation for milk and fruits and vegetable juices and specially for tomato juice.

Check Your Progress Exercise 3

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

1. Name the scientist who invented Pasteurization. In which food material this process is used the most?

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2. Why pasteurization of milk is important?

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3. What are the factors responsible for microbial inactivation during pasteurization?

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4. Does pasteurization kill all the bacteria in the product?

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5. Name the organisms on which milk pasteurization time and temperature is based?

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6. Why HTST pasteurization is better treatment than LTLT?

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6.5 PRESERVATION BY MOIST HEAT

Moisture levels of the food material are a definite influencing factor in the shelf life of food. Moist heat readily kills viruses, bacteria, and fungi by

denaturing enzymes whereas dry heat kills by oxidation of cell contents. There is a correlation between the percent of water and the effectiveness of heat to kill microorganisms. Moist heat is a more effective sterilizing agent than dry heat because the moisture increases the rate of heat penetration. Moist heat requires less heat (temperature or time) than dry heat (121°Cfor 10 min of moist heat is equivalent to about 30 min at 200°C dry heat).

For this reason a lot of sterilization procedures use super heated steam that provides moist heat. Temperature over 100^0C requires heating under elevated pressure, (like in a pressure cooker) 121^0C require 100 kpa extra pressure. It is important that no air pockets are allowed to develop when a product being sterilized with steam. In air pockets food is exposed to dry heat and thus the time /temperature is not enough. Moist heat denatures proteins which destroys essential enzyme activities.

Endospores are much more resistant to heat than are vegetative cells. For this reason, moist heat sterilization is aimed at ensuring that endo spores are killed.

Terms Associated with Heat Preservation

Scientists use different terms to refer the effect of moist heat on the preservation of food. These terms include thermal death time, D-value, and z-value.

6.5.1 Thermal Death Time (TDT)

Thermal death time is the amount of time that is necessary to kill a specific number of microbes at a specific temperature. This value is obtained by keeping temperature constant and measuring the time necessary to kill the amount of cells specified.

6.5.2 D-Value

The term D-value refers to decimal reduction time. This is the amount of time that it takes at a certain temperature to kill 90% of the organisms being studied. Thus after an organism is reduced by 1 D, only 10% of the original organisms remain. The population number has been reduced by one decimal place in the counting scheme. When referring to D-values it is proper to give the temperature as a subscript to the D. For example, a hypothetical organism is reduced by 90% after exposure to temperatures of 149°C for 2 minutes, Thus the D-value would be written as D_{300F} = 2 minutes. Several parameters help us to do thermal calculations and define the rate of thermal lethality. The D-value is a measure of the heat resistance of a microorganism. It is the time in minutes at a given temperature required to destroy 1 log cycle (90%) of the target microorganism. (Of course, in an actual process, all others that are less heat tolerant are destroyed to a greater extent). For example, a D-value at 72°C of 1 minute means that for each minute of processing at 72°C the bacteria population of the target microorganism will be reduced by 90%. D-values vary according to the temperature, species of microorganisms, number of initial population, and other factors that may affect thermal resistance. In the illustration below, the D-value is 14 minutes (40-26=14 min.) and would be representative of a process at 72°C.

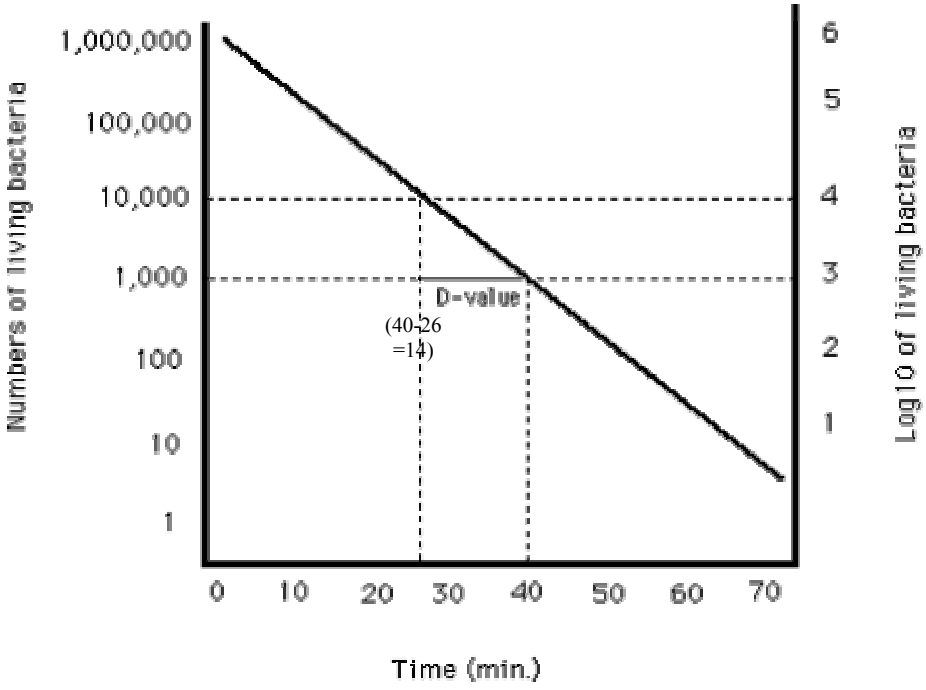

Figure 6.1: The D-value

6.5.3 Z-Value

The Z-value reflects the temperature dependence of the reaction. It is defined as the temperature change required to change the D-value by a factor of 10. While the D-value gives us the time needed at a certain temperature to kill an organism, the Z-value relates the resistance of an organism to differing temperatures. In the illustration below the Z-value is 10°C.

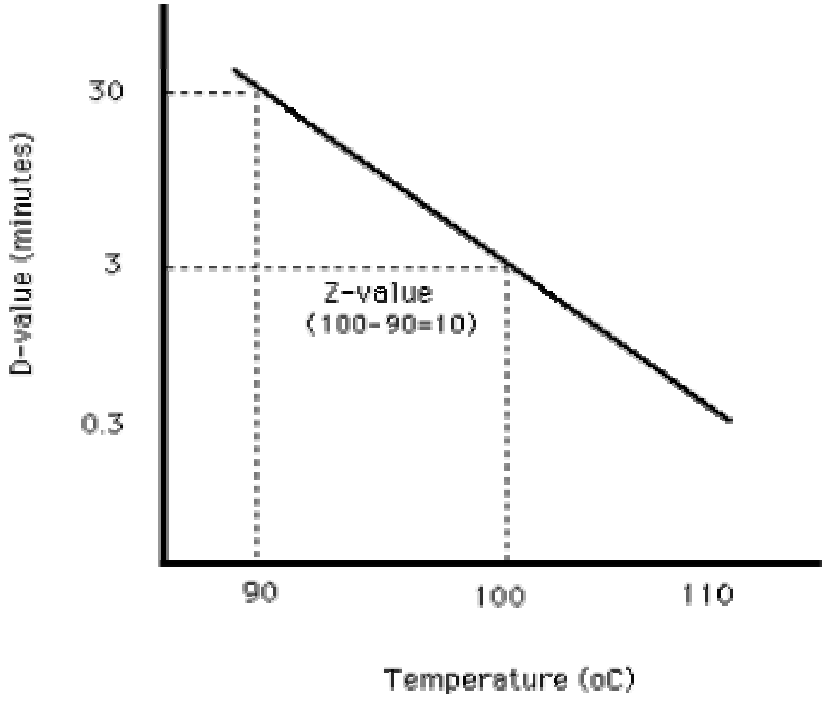

Figure 6.2: The Z-value

The Z-value allows us to calculate a thermal process of equivalency, if we have one D-value and the Z-value. So, if it takes an increase of −12°C to move the curve one log, then our Z-value is 10. So then, if we have a D-value of

4.5 minutes at 66°C, we can calculate D-values for 71°C by reducing the time by 1 log. So, our new D-value for 71°C is 0.45 minutes. This means that each -12°C increase in temperature will reduce our D-value by 1 log. Conversely, a -12°^{0}C decrease in temperature will increase our D-value by 1 log. So, the D-value for a temperature of 60°^{0}C would be 45 minutes.

Reactions that have small Z-values are highly temperature dependent, whereas those with large Z-values require larger changes in temperature to reduce the time. A Z-value of 10°C is typical for a spore forming bacterium. Heat induced chemical changes have much larger Z-values that microorganisms, as shown below:

	Z (°C)	**D121(min)**
bacteria	5-10	1-5
enzyme	30-40	1-5
vitamins	20-25	150-200
pigment	40-70	15-50

Figure 6.3 illustrates the relative changes in time temperature profiles for the destruction of microorganisms. Above and to the right of each line the microorganisms or quality factors would be destroyed, whereas below and to the left of each line, the microorganisms or quality factors would not be destroyed. Due to the differences in Z values, it is apparent that at higher temperatures for shorter times, a region exists (shaded area) where pathogens can be destroyed while vitamins can be maintained. The same holds true for other quality factors such as colour and flavour components. Thus in milk processing the higher temperature, shorter time (HTST) process (72°C/16 sec) is favoured compared to a lower temperature longer time (batch or vat) process since it results in a slightly lower loss of vitamins and better sensory quality.

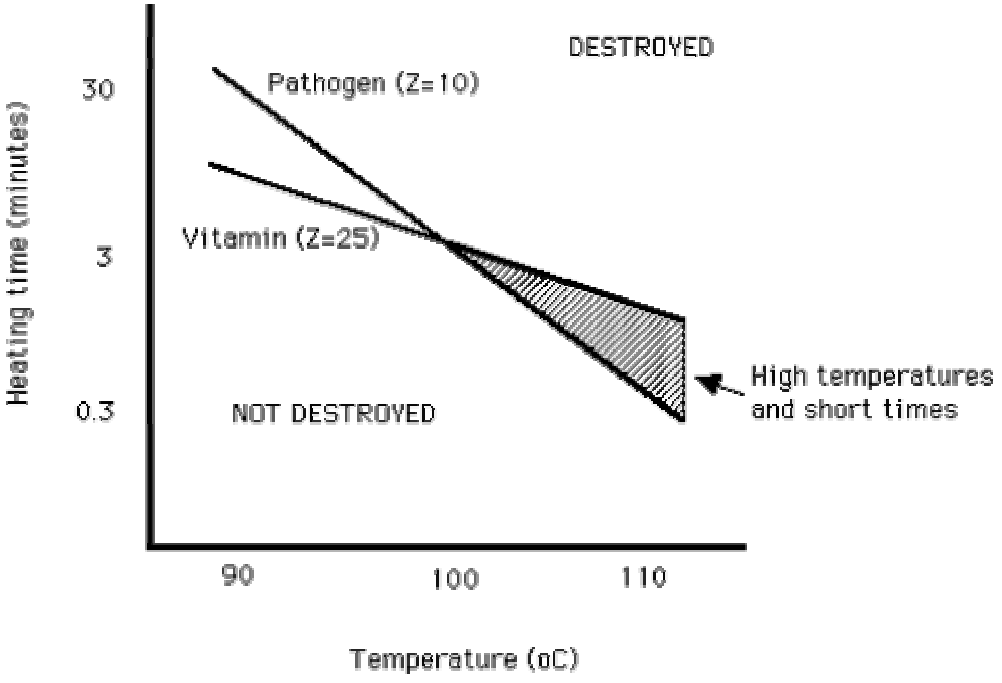

Figure 6.3: The relative changes in time temperature profile

Alkaline phosphatase is a naturally-occurring enzyme in raw milk which has a similar Z value to heat-resistant pathogens. Since the direct estimation of pathogen numbers by microbial methods is expensive and time consuming, a simple test for phosphatase activity is routinely used. If activity is found, it is assumed that either the heat treatment was inadequate or that unpasteurized milk has contaminated the pasteurized product.

6.5.4 12-D Concept

Canned foods are susceptible to the spores of the organism *Clostridium botulinum*. This is the organism that causes botulism. Their bacterial spores can survive many heat treatment processes. However, in modern food production, canned foods are subjected to a time/temperature process that will reduce the probability of the survival by the most heat-resistant *C. botulinum* spores by 12 logs or 12-D at 250F (the temperature used in the calculation of most commercial 12-D processes is 250F, and the D-value for this organism at 250F is 0.21 minutes). This process is based on the assumption of the number of surviving spores in one can. If it is assumed that a container had one million spores per can the heat treatment needed to reduce the number to one in one million i.e. from 10^6 to 10^{-12} involves a reduction of twelve decimal places i.e. from 1,000,000 to 0.0000001

6.5.5 F-Value

If we assume that there are 10 surviving spores in one can, then we can calculate the time for a 12-D process to occur by using the following formula:

- $F_0 = D_{250F}$ (log a − log b), where a = initial population and b = final population.
- So F_0 = (0.21min.) (log 10^1 − log 10^{-11}), we move down 12 log values (1 − (−11)) = 12.
- So, F_0 = (0.21min.) (1 − (−11)), or 0.21 × 12 = 2.52 minutes.

Simply put, (D-value at 250F) × (12) results in a 12-D process.

The killing effect of a time / temperature combination is referred to as the **F-value.**

F = 1 is heat killing effect equivalent to 1 min at 121°C.

The F-value required to achieve a 12D cook depends on the resistance of the particular type of bacteria. One of the most resistant species is *Bacillus stearothermophilus* which is 5 or 6 time more resistant than *C. botulinum.*

A 12-D cook for *Cl. botulinum* may require an F value of 2.52
A 12-D cook for *B. stearothermophilus* may require F = 18

From food safety angle, the microorganisms of greatest concern are *Salmonella* sp., *Clostridium perfringens*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter sp*., and *E. coli*, all of which have much lower z values and consequently should achieve a 12D process in a shorter time. *Bacillus* of the most heat resistant strains of bacteria known.

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. Why moist heat is a more effective sterilizing agent than dry heat?

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2. Define D-value and Z-value. How these terms are inter-related?

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3. What is the principal of 12D concept?

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4. Define F-value?

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6.6 MICROBIOLOGY OF THERMALLY PROCESSED FOOD

As you can now very well understand the heat is an important way of preserving foods. Still some thermally processed foods undergo spoilage due to chemical or biological reasons. The most important chemical spoilage of canned foods is the hydrogen swell produced as a result of action of food acids with the metals. Biological spoilage of thermally processed foods by microorganisms may result either from the survival of organisms after the heat treatment or leakage of the container permitting entrance of the microorganisms. Surviving organisms may be vegetative cells or spore formers depending upon the heat treatment. Acid foods are processed at temperature around $100^{O}C$ which result in the killing of all vegetative cells of bacteria, yeasts and molds. Only bacterial spores may survive *stearothermophilus* is a non-pathogenic organism that has been shown to be one but these do not grow in acid foods. On the other hand, meat, vegetables and milk are processed at low temperatures. This may eliminate vegetative cells but not the spores, which germinate later and cause spoilage. Microorganisms that enter through leaks during cooling need not necessarily be heat resistant.

6.6.1 Spoilage by Thermophilic Bacteria

Under processing of low acid foods result in spoilage by thermophilic (microorganisms which require high temperature, more than 45°C for their growth) bacteria such as *Bacillus coagulance, Bacillus stereothermophilus*. These microbes produce heat resistant endospores than can survive 121°C for 4-5 minutes. These organisms produce acid without gas. This is known **as flat sour spoilage**. Some times In Low and medium acid foods the cans swell due to production of carbon dioxide and hydrogen by *Clostridium thermosaccharolyticum*. This is known as **thermophilic spoilage**. **Sulphide spoilage** is caused by *Clostridium nigrificans* in low acid foods. Spores of this bacterium are not very heat resistant and their presence is indicative of under processing. Spoilage is indicated by the the presence of H_2S and blackening of material. Sources of all these material are generally, the plant equipment, sugar, starch, soil etc.

6.6.2 Spoilage by Mesophilic Organisms

Mesophilic microorganisms are those microorganisms which grow best at temperature 25-45°C. Spoilage of canned foods by mesophilic organisms is indicative of under processing and is caused by species of Bacillus, Clostridium, Yeast and fungi. *Clostridium butyricum* and *C. pasteurianum* produce a butyric acid type of fermentation in acid or medium acid foods with swelling of the container by the production of CO_2 and H_2. Other species of *Clostridia* may produce H_2S causing can to swell. These putrefactive anaerobes (Micro organism that grow in the absence of oxygen) generally grow in low acid foods such as peas corn, meat, poultry etc. but some times may also spoil medium acid foods.

Some *Bacilli* such as *Bacillus subtilis* and *B. mesentroides* have been found to grow in poorly evacuated cans of sea foods, meat and milk. The gas forming Bacilli (B.polymyxa, B. macerans) are also reported to cause spoilage of canned peas, spinach, peaches and tomatoes.

The presence of non spore forming bacteria in canned food is an indicative of leak or under processing. *Streptococcus thermophilus*, *Pseudomonas*, *Micrococcus* and *proteus* have also been reported to cause spoilage of thermally processed products. Molds, yeast and their spores are destroyed at pasteurization temperature. Their presence is indicative of under processing or leakage. Spoilage of canned fruits and fruit products by yeasts may result in CO_2 production and spoilage of cans. Film yeast and fungi grow on the surface and cause degradation of the product.

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. What are the probable reasons for biological spoilage of thermally treated foods?

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2. Name two thermophilic microorganisms responsible for flat sour spoilage.

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3. What is thermophilic spoilage?

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4. What do you understand about sulphide spoilage of low acid foods?

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5. Name some mesophilic bacteria responsible for spoilage of thermally treated foods.

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6.7 LET US SUM UP

Thermally processed foods are those that have been i) heated in hermetically sealed container ii) have been filled hot into a container which is then closed and cooled. The purpose of these processes is to destroy pathogenic microorganisms and those that might grow and cause spoilage of the particular food. The food that are commercially sterile are those that will not support microbial growth when exposed to the usual temperatures during storage, transport and marketing. However, they may not be completely free of microorganisms. Pasteurization is heat treatment to inactivate some microorganisms. Thermally processed foods may get spoiled due to under processing or leakage.

6.8 KEY WORDS

Thermophilic : Microorganisms which grow at temperature above 45°C.

Mesophilic : Microorganisms which grow at temperature 25-45°C.

Under processing : Lower time or temperature treatment.

6.9 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check You Progress Exercise 1

1. Your answer should include the following points:

   - Microorganisms that spoil product are typically called **spoilage organisms**.
   - Microorganisms that can make people sick are **pathogens**.

2. Your answer should include the following points:

   - to keep the food in a stable condition over a period of time
   - to prevent it from spoilage or making people sick.

3. Your answer should include the following points:

   - chemical preservation
   - modified atmospheres
   - irradiation
   - low temperature preservation
   - preservation by drying
   - high temperature preservation.

4. Your answer should include the following points:

   - Time-temperature combination
   - heat penetration characteristics of particular food
   - the type of micro-organisms present in the food material
   - the thermal death time of different microorganisms
   - type of food
   - concentration of the microorganisms
   - state of the microorganism
   - effect of heat on the product
   - the degree of heat penetration
   - food acidity / pH value.

Check You Progress Exercise 2

1. Your answer should include the following points:

   - complete destruction of micro-organisms.

2. Your answer should include the following points:

   - the condition achieved by the application of heat sufficient to render the processed product free from viable microorganisms
   - capable of growing in the food under normal non-refrigerated temperatures at which the food is likely to be held during distribution and storage

- Unlike sterilization here the food is not completely free of microorganisms.

Check You Progress Exercise 3

1. Your answer should include the following points:

   - process created by Louis Pasteur
   - aimed to destroy bacteria, molds, spores etc.
   - discovery about the destruction of bacteria by exposing them to certain minimum temperature for certain minimum time
   - the higher the temperature the shorter the exposure time required.
   - process applied to milk.

2. Your answer should include the following points:

   - Public Health Aspect – to make milk and milk products safe for human consumption by destroying all bacteria that may be harmful to health (pathogens)
   - Keeping Quality Aspect – to improve the keeping quality of milk and milk products. Pasteurization can destroy some undesirable enzymes and many spoilage bacteria. Shelf life can be 7, 10, 14 or up to 16 days.

3. Your answer should include the following point:

   - extent of microbial inactivation depends on the combination of temperature and holding time.

4. Your answer should include the following points:

   - Through pasteurization all of the bacteria are not completely destroyed, it still exists in pasteurized products, but in very low concentrates.
   - Refrigeration keeps the bacteria from further growth, very low.

5. Your answer should include the following points:

   - thermal death time studies for the most heat resistant pathogens found in milk
   - *Coxiella burnetii* and *Mycobacterium tuberculosis* are the most heat resistant non spore forming pathogens.

6. Your answer should include the following points:

   - bacterial destruction is very nearly equivalent in LTLT and in HTST pasteurization processes but
   - HTST treatment give the best quality products in respect of flavour and vitamin retention.

Check You Progress Exercise 4

1. Your answer should include the following points:

   - moisture increases the rate of heat penetration.
   - Moist heat requires less heat (temperature or time) than dry heat
   - 121°C for 10 min of moist heat is equivalent to about 30 min at 200°C of dry heat.

2. Your answer should include the following points:

   - D-value is the amount of time that it takes at a certain temperature to kill 90% of the organisms being studied.
   - Z value is defined as the temperature change required to change the D-value by a factor of 10.
   - D-value gives us the time needed at a certain temperature to kill an organism
   - Z-value relates the resistance of an organism to differing temperatures.

3. Your answer should include the following points:

   - process based on the assumption of the number of surviving spores in one can.
   - canned foods subjected to a time/temperature process that will reduce the probability of the survival of the most heat-resistant *C. botulinum* spores by 12 logs i.e. from 1,000,000 to 0.0000001.

4. Your answer should include the following point:

   - killing effect of a time / temperature combination.

Check You Progress Exercise 5

1. Your answer should include the following point:

   - Under processing or leakage may be the cause of spoilage of thermally treated foods.

2. Your answer should include the following point:

   - *Bacillus coagulance* and *Bacillus stereothermophilus.*

3. Your answer should include the following point:

   - In Low and medium acid foods the cans swell due to production of carbon di oxide and Hydrogen by *Clostridium thermosaccharolyticum*.

4. Your answer should include the following points:

   - caused by *Clostridium nigrificans* in low acid foods.
   - Spores of *Clostridium nigrificans* are not very heat resistant and their presence is indicative of under processing.

- Spoilage is indicated by the the presence of H_2S and blackening of material.
- Sources of all these material are generally, the plant equipment, sugar, starch, soil etc.

5. Your answer should include the following points:

- *Clostridium nigrificans*
- *C. pasteurianum*
- *Bacillus subtilis*
- *B. mesentroides*
- *B.polymyxa*
- *B. macerans*
- *Streptococcus thermophilus*
- *Pseudomonas*
- *Micrococcus* and *Proteus.*

6.10 SOME USEFUL BOOKS

1. Adams, M.R. and Moss, M.O. (2000) Food Microbiology. Royal Society of Chemistry, Cambridge, U.K.

2. Jay, J.M. (2000) Modern Food Microbiology, Van Nostrand Company, New York.

UNIT 7 DRYING – CONTROLING OF MICROORGANISMS

Structure

7.0 OBJECTIVES

After studying this unit, you should be able to:

- know the importance of dehydrated fruits and vegetables;
- have an idea about the drying/dehydration theories of fruits and vegetables;
- have the knowledge about the microorganisms evolved in dried products;
- know the different kinds of microorganism associated with dried foods; and
- know about the microbial spoilage of dried fruits and vegetable products.

7.1 INTRODUCTION

Drying or dehydration is accomplished by the removal of water from the fruits and vegetables below a certain level at which enzyme activity and growth of microorganisms is affected adversely. The dried fruits and vegetable are called as high sugar high acid foods or high value low volume foods. These dried or concentrated products save energy, money and space in shipping, packaging, storing and transportation. Dehydration or drying process usually involves heating, in which water is removed from solid or near solid substances. The term **drying** is generally used for drying of the produce under the influence of non-conventional energy sources like sun and wind. **Dehydration** on the other

hand refers to the process of removal of moisture by the application of artificial heat under controlled conditions of temperature, relative humidity and air flow. The sun drying is a slow process and thus, not suitable for many high quality products. Generally, it lowers the moisture contents below about 15% which is too high for storage stability of numerous products.

7.2 PRINCIPLES

The basic principle in the process of drying or dehydration is the removal of sufficient moisture to protect the product from spoilage. The process reduces the amount of available moisture i.e. the water activity (a_w) and hence, product becomes shelf-stable and is preserved for quite a long period. Moisture-solid, relationship in fruits and vegetables are more complex than in inorganic materials as the matter in fruits and vegetables exhibits an energetic retention of moisture and the moisture is bound to the solid. The solid skeleton consists essentially of numerous cells joined together to provide a network of capillaries, some of them are very fine. First the moisture in the larger capillaries has to be evaporated then only the moisture in the finer capillaries can be removed. The cell walls act as semi-permeable membranes for the diffusion of moisture which is mainly held osmotic ally. Finally, there is a small amount of moisture adsorbed on the skeletal frame in multi molecular layers. In order to dehydrate any product specific requirements need to be fulfilled so that the product retains as much as possible, its original characteristics.

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 do you mean by drying and dehydration?

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2. What are the main objective of drying?

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3. What is the role of air in the process of drying or dehydration?

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4. How the fruits & vegetables are more complex than inorganic?

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7.3 MECHANISM OF DEHYDRATION

The changes during dehydration can be largely explained in terms of heat and mass transfer phenomena. A cue of food in the course of dehydration loses moisture from its surface and develops dried layer with remaining moisture confined to its centre. From the centre to the surface a moisture gradient will be stabilized. The outside dried layer acts as an insulation barrier against rapid heat transfer into the food pieces, this is further decreased by air voids formed by evaporating water. In addition to less driving force from decreased heat transfer, the centrally remaining water also now has further to travel to get out of the food piece than did surface moisture at the start of drying. In addition, as the food dries it approaches its normal equilibrium relative humidity, as it does; it begins to pick up molecules of water vapour from the drying atmosphere as fast as it loses them. When these rates are equal drying ceases.

7.4 THEORY OF DRYING

There are two steps involved in drying & dehydration.

7.4.1 Heat Transfer Theory

Transfer of heat consists of transferring of molecular or atomic motion from one region to another. There are three broad mechanisms by which such transfer can occur, conduction, convection, and radiation. In conduction, the energy is transmitted from particle to particle by a process of direct contact. Transfer of heat by convection involves bulk mixing of fluids of different temperatures. Radiation is the transfer of energy from a radiating source through space which may or may not be occupied by matter. It is by radiation that we receive all our energy from the sun.

7.4.2 Mass Transfer Theory

The removal of moisture from a food product involves simultaneous heat and mass transfer. Heat transfer occurs within the product structure and is related to the temperature gradient between the product surface and the water surface at some location within the product. As sufficient thermal energy is added to the water to cause evaporation, the vapours are transported from the water

surface within the product to the product surface. The gradient causing moisture –vapour diffusion is vapour pressure at the liquid water surface, as compared with the vapour presser of air at the product surface. The heat and the mass transfer within the product structure occurs at the molecular level, with heat transfer being limited by thermal conductivity of the product structure, while mass transfer is proportional to the molecular diffusion of water vapour in air. The rate of moisture diffusion can be estimated by the expression for molecular diffusion. The mass flux for moisture movement is a function of the vapour pressure gradient as well as the mass diffusion for water vapour in air, the distance for water vapour movement within the product structure and temperature. The transport of vapour from the product surface to the air and the transfer of heat from the air to the product surface is a function of the existing vapour pressure and temperature gradients, respectively, and the magnitude of the convective coefficient at the product surface.

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. In the food product how the changes are occurred.

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2. What are the main mechanisms by which heat can transfer?

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3. How the evaporation is occurred during drying?

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4. What are the functions of vapour pressure and temperature gradient?

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7.5 IMPORTANCE OF WATER ACTIVITY (a_w)

Water activity (a_w) is defined as the ratio of the vapour on the aqueous solution to that of pure water at the same temperature i.e.

$$a_w = \text{Vapour pressure of solution at } T^oC$$

Vapour pressure of pure water at T^oC.

Water activity is also equal to the equilibrium relative humidity (ERH);

$$a_w = \frac{\text{Equilibrium relative humidity}}{100}$$

The a_w has a major role to play in microbiological spoilage and chemical changes produced in the food. The principles of water and microorganisms relation includes:

1) Water activity, rather than water content, determines the lower limit of available water for microbial growth. Most bacteria do not grow below a_w 0.91 and most molds cease to grow at water activity of 0.80. Some xerophylic fungi have been reported to grow at water activities of 0.65, but the range of 0.70 – 0.75 is generally considered their lower limit.

2) Environmental factors affect the level of water activity required for microbial growth. The less favourable the other environmental factors (nutrients, pH, oxygen pressure, temperature) the higher becomes the minimum a_w at which microorganisms can grow.

3) Some adaptation to low water activities occurs, particularly when a_w is depressed by addition of water soluble substances (principle of IMF – Intermediate Moisture Food), rather than by water crystallization (frozen foods) or water removal (dehydrated foods).

4) When water activity is depressed by solutes. The solutes themselves may have effects which complicate the effect of a_w per se. For instance, at a given a_W microbial growth is less effectively depressed by glycerol than by sodium chloride. More recent (IMF – Intermediate Moisture Food) have resulted in the following additional findings.

   a) Water activity modifies sensitivity of microorganisms to heat, light and chemicals. In general organisms are most sensitive at high water activities (i.e. in dilute solution) and minimum sensitivity occur in an intermediate moisture. Minimum water activities for production of toxins are often higher than those for microbial growth. The phenomenon may represent an important safety factor in the distribution of dehydrated and intermediate moisture foods.

   b) The effect of water on chemical reactions in foods are more complicated than are its effect on microbial growth. It plays one or more of the following roles; a) as a solvent for reactant and for products, b) as a reactant (e.g. in hydrolysis reactions) c) as a product of reactions and d) as a modifier of the catalytic or inhibiting activities of other substances (e.g. water in activities some metallic catalysts of lipid per oxidation).

All microorganisms have an optimal and minimal water activity for growth. Adjusting the a_w of a product by addition of solutes or the removal of water, to a point below the minimal a_w of the normal spoilage flora results in a microbiological stable product. Many of the products contain viable microorganisms and spores, which are not able to germinate because of the restrictative a_w. In fabrication of a product with a reduced a_w other factors which would affect the growth of microorganisms present need to be considered, since the a_w on microorganisms is influenced by pH, oxygen level, temperature, nutrient content, and possibly food preservative, either natural or added.

Water activity (a_w) influences the physical, chemical and microbiological properties of many substances. The shelf life of foods, their colour, stability, taste, texture, vitamin content, aroma, mold formation and microbiological growth properties are influenced directly by the a_w value. a_w measurement is required to meet standards like FDA – Food Drug Act, USDA – United State Department of Agriculture, GMP – General Manufacturing Practices, HACCP – Hazard Analysis and Critical Control Points, and BIS 15000 – Bureau of Indian Standards: The foods types and range of a_w is discussed as given below.

A_w range	**Upper limit values for micro-organisms**	**Foods in this range**
1.00-0.95	*Pseudomonas, Escherichia, Proteus, Shigella, Clesiella, Bacillus, Clostridium, perfringens,* some yeast	Perishable (fresh) food and fruit in tins, Vegetables, meat, fish and milk, cooked sausage, backed bread, food with a content up to 40% weight sucrose or 7% common salt.
0.95-0.91	*Salmonella, Vibrio parahaemoliticus, C. botulinum,* Serratia, *Lactobacillus, Pediococcus,* some mold, yeast	Some cheese (cheddar, Swiss, Muenster, and Provolone) smoked meat (ham) some fruit juice concentrates, food with a 55% weight sucrose (saturated) or 12% common salt.
0.91-0.87	Many types of yeast *(Candida, Torulopsis, Hansenula), Micrococcus*	Matured sausages (salami), cake, dry chesses, margarine, and food with a 65% weight sucrose (saturated) or 15% common salt.
0.87-0.80	Most types of mold (mycotoxic *Penicillia), Staphylococcus aureus,* most *Saccharomyces* (bialii) spp. , *Deboryamyces*	Most fruit juice concentrates, sweetened milk, chocolate syrup, maple and fruit syrup, flour, rice, pulses with a water content 15-17%, fruit cakes traditional smoked hams.
0.80-0.75	Most types hallophilic bacteria, mycotoxic aspergilli	Marmalade, jam, fruit jelly, marzipan, glace fruit, some types of marshmallow.
0.75-0.65	Xerophylic mold *(Aspergillus chevalier, A. candid us, Wallemia semi), Saccharomyces bisporus*	Rolled oats with a 10% water content, naught, fondant, marshmallows, grouts, molasses, raw sugar, some dried fruit, and nuts.

<table>
<tr><td>0.65-0.60</td><td>Osmophyilc yeast (Saccharomyces rouxi), some mould (Aspergillums echinulatus, Monascus bisporus)</td><td>Dried fruit with 15-20% water content, some types of toffee, caramel, honey</td></tr>
<tr><td rowspan="2">0.5</td><td rowspan="5">No microbial growth</td><td>Noodles, Spaghetti, pasta. etc. with about 12% water content, spices with about 10% water content</td></tr>
<tr><td rowspan="2">Egg powder with about 5% water content</td></tr>
<tr><td>0.4</td></tr>
<tr><td rowspan="2">0.3</td><td>Biscuits, crackers, bread crust, cookies, etc. with about 3-5% water content</td></tr>
<tr><td rowspan="2">Powder milk with about 2-3% water content, dried vegetables/ fruit with about 5% water content, cornflakes with about 5% water content fruit cake, rustic, crackers, biscuits</td></tr>
<tr><td>0.2</td></tr>
</table>

Check Your Progress Exercise 3

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

1. Name some of products which have the a_w between 0.65-0.60.

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2. Name the microorganisms which are found at a_w between 0. 65-0.60.

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7.6 MICROORGANISMS ASSOCIATED WITH DRIED FRUITS AND VEGETABLES

Microorganisms are associated, in a variety of ways, with all of the food we eat. They may influence the quality, availability, and quantity of our food. Naturally occurring foods such as fruits and vegetables normally contain some microorganisms, and may be contaminated with additional organisms during handling. Food serves as a medium for the growth of microorganisms, and this growth may cause the food to undergo decomposition and spoilage. Food may

also carry pathogenic microorganisms and as a result transmit diseases. Dried foods has been used for centuries and they are more common throughout the world than frozen foods. Growth of all microorganisms can be prevented by reducing the moisture content of their environment below a critical level. The critical level of moisture is determined by the characteristics of the particular organisms and the capacity of the food item to bind water so that it is not available as free moisture.

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. In what way microorganisms affect the products.

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2. How the growth of microorganisms can be arrested.

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7.7 MICROBIOLOGY OF FRUITS AND VEGETABLE

7.7.1 Microbiology of Fresh Fruits and Vegetables

Fruits and vegetables are normally susceptible to infection by bacteria, fungi and viruses. Microbial invasion of plant tissue can occur during various stages of fruits and vegetables development and hence to the extent that the tissues are infected the likelihood of spoilage is increased. A second factor contributing to the microbial contamination of fruits and vegetables pertains to their post harvest handling. Mechanical handling is likely to produce breaks in the tissue which facilitates invasion by microorganisms. The pH of fruits is relatively acid ranging from 2-3 for lemons to 5.0 for bananas. This resists bacterial growth but does not retard fungal growth. The range for vegetables is slightly higher pH 5.0 to 7.0 and hence they are more susceptible than fruits to attack by bacteria.

7.7.2 Microbiology of Dried Fruits and Vegetables

The microorganisms on most of the dried fruits vary a few hundred per gram of fruits to thousands, and in whole fruits they are mostly on the outer

surfaces. Spores of bacteria and molds are likely to be most numerous. When part of the fruit has supported growth and sporulation of mold before or after drying, mold spores may be present in large numbers. The number on the vegetable just before drying may be high because of contamination and growth after blanching and the percentage killed by the dehydrating process usually is less than with the more acid fruits. If drying trays are improperly loaded, souring of such vegetables as onions or potatoes by lactic acid bacteria with marked increase in number of bacteria which may take place during the drying process. The risk may be grater of the fruits and vegetables which are not blanched before drying like onion. Microbial counts on dried vegetables range from negligible to millions per gram. A number of genera of bacteria found on dried vegetables includes: *Escherichia, Enterobactor, Bacillus, Clostridium, Micrococcus, Pseudomonas, Streptococcus, Lactobacillus and Leuconostoc*. Of these, *Lactobacillus* and *Leuconostoc* species are predominant in many samples of dehydrated vegetables.

Dried fruits become musty of molds and dried vegetables soft or slimy if kept in a damp atmosphere in unsealed containers. Hence, proper sealing and storing of containers at ambient temperature and in a dry place is important. Dried fruits and vegetables should be packed in moisture proof containers. Higher density polythene (HDP) package of multiple aluminium foil are utilized for packing of dried vegetables.

The bacteria, such as *Bacillus* sp., *Clostridium* sp., *Micrococcus* sp, *Streptococcus* sp., and *Pseudomonas* sp. are common as soil and water born. Bacteria capable of causing food poisoning such as *Salmonella* sp. and *Clostridium botulinum*, are not found in dehydrated foods as in case of dehydrated onion where the microbial load is influenced by the following factors:

1. The load and types of microorganism present on the raw material.
2. Pre-treatment given to the material
3. Time lag between preparation and dehydration
4. Drying time and temperature
5. Moisture content of the finished product
6. In plant sanitation,
7. Packaging and storage conditions of the finished product.

One of the important types of microbiological spoilage in onion during dehydration is fermentation and souring, which are undesirable and make the product sub-standard. Sour onions have characteristics sour taste commonly associated with vegetable tissues undergoing lactic acid fermentation. Onion slices do not dry properly. Pink discolouration and off taste are indications of spoilage. Bacteria which predominate in fresh onions include representatives of the genera: *Lactobacillus*, and *Aerobactor*. Fresh onions juice sterilized by filtration suppressed the growth of *Bacillus* subtilis and *E. coli*. but did not prevent the growth of *Lactobacillus brevis* and *Aerobactor aerogens*. In good quality of dehydrated onion many aerobic bacilli and other soil and water born bacteria are not found which may be due to the toxicity of constituents present in fresh onions.

To check the souring and fermentation as well as to reduce the microbial load to the minimum the following points should be closely watched:

1. Onion bulbs selected for dehydration should be free from disease and blemish

2. Onions should be thoroughly washed after peeling in 3-5 ppm chlorine water.

3. The cut slices should be dried immediately under controlled conditions so that the finish product can be obtained in the minimum time having moisture at 6-7 percent.

4. Sanitary conditions and workers hygiene in the factory should be controlled and

5. Proper packing room facilities and nitrogen gas packaging are important for the storage of finished product

The number of microorganisms and their kinds vary at different stages of processing such as

7.7.3 Before Reception at the Processing Plant

The microbiology of foods before their reception at the processing plant is likely to be similar whether the foods are to be dried, chilled, frozen, canned or otherwise processed. Fruits and vegetables have soil and water organisms on them when harvested, plus their own natural surface flora and spoiled parts contain the microorganisms causing the spoilage. Growth of some of these organisms may take place before the foods reach the processing plant if environmental conditions permit. Thus piled vegetables may raise temperature and stimulate the growth of slime- forming, flavour harming, or even rot-producing organisms.

7.7.4 In the Plant before Drying

Growth of microorganisms that begun on foods before they have reached the drying unit may continue up to time of drying. Also equipment and workers may contaminate the food. Some of the pre-treatment reduce number of organisms while other may increase them, but the foods may be contaminated after these treatments. The grading, selection, and sorting of fruits and vegetables, influences kinds and number of microorganisms. The elimination of spoiled fruits and vegetables or of spoiled parts reduced number of organisms in the product to be dried.

Washing of fruits and vegetables removes soil and other adhering materials and serves to remove microorganisms. There also possibility of adding organisms if the water is of poor quality.

Peeling fruits or vegetables, especially with steam or lye, and slicing or cutting reduces the number of organisms if equipment is adequately cleaned and sterilized.

Dipping in dilute alkali as applied to certain fruits before sun drying may reduce the microbial population.

Blanching or scalding vegetables reduces the bacterial numbers greatly, as much as 99 percent in some instances. Sulphuring of fruits and vegetables also causes a great reduction in number of microorganisms and serves to inhibit growth in the dried product.

7.7.5 During the Drying Process

Heat applied during a drying process causes a reduction in total number of microorganisms, but the effectiveness varies with the kinds and numbers of organisms originally present and the drying process employed. Usually all

yeasts and most bacteria are destroyed, but spors of bacteria and molds commonly survives, as do vegetative cells of a few species of heat resistant bacteria, improper conditions during drying may even permit the growth of microorganisms. More microorganisms are killed by freezing than by dehydration during the freezing- drying process.

7.7.6 After Drying

If the drying process and storage conditions are adequate there will be no growth of microorganisms in the dried foods. During storage there is a slow decrease in number of organisms, the microorganisms that are resistant to drying will survive best: therefore the percentages of such organisms will increase. Especially resistant to storage under dry conditions are the spores of bacteria and molds, some of the micrococci, and micro bacteria. There may be some opportunity for contamination of the dried food during packaging and other handling subsequent to drying.

Special treatment given to some dry foods will influence microbial numbers e.g. sweating of dry fruits to equalize moisture may permit some microbial growth. Pasteurization of dry fruits will reduce number of microorganisms. Some products are re-packaged for retail sale, e.g. figs in the near east, are subjected to contamination. The microbial content and the temperature of water used to rehydrate for dried foods also affect the number of microorganisms if rehydration done in water at 50°C the number of microorganisms will be more and number of microorganisms is almost eliminated when the product is rehydrated at 85 to 100°C.

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. Write the name of microorganisms which spoil attack to the fruits and vegetables.

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2. What are the factors which affect the microbial load in the finished products?

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3. Write few points which help to check the incidence of souring, fermentation, and microbial load.

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4. What are the factors which reduce the microorganisms in fresh fruits and vegetables?

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5. Write few treatments which given to dehydrated fruits for control of microorganisms.

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7.8 SURVIVAL OF MICROORGANISMS IN DRIED FOODS

The survival of microorganisms in dried foods can be markedly affected by a_w level. There are important interaction between a_w and such factors as pH, oxygen and food composition. For many foods, deterioration during storage in the dry state is least at the relatively low a_w levels. Survival of pure cultures of vegetative bacteria equilibrated to a range of a_w levels after freeze drying have shown clearly the increase in survival that accompanies reduction in a_w level to 0.1 -0.2. Survival of *Salmonella newport* at 0.0 a_w, after freeze –drying in papain digest, was nearly maximum in vacuum, but was very poor when stored in air. Although *Pseudomonas fluorescense* proved more susceptible to death on storage, the qualitative response to a_w was similar. Death of bacteria during storage at reduced a_w levels is greatly influenced by the nature of solution from which they had been dried.

7.8.1 Survival at Freezing Temperatures

Freezing and frozen storage may reduce greatly the viability of populations of sensitive microorganisms. The latter include the vegetative cells of yeasts and molds and most gram negative bacteria. Gram positive bacteria, especially cocci, are more resistant, and for these reasons enterococci are frequently claimed to be more suitable than *Escherichia coli* as indicators of fecal contamination in frozen foods. Many fungal spores also show this level of resistance, however bacterial spores are least affected by freezing.

The rate of freezing influences the survival, because of it influence the size of ice crystals and hence the degree of mechanical damage caused to cellular structures. Rapid freezing is less damaging than slow freezing. It is the range of temperature between freezing point of a food and its eutectic, influences the a_w of food, but not its overall solute concentration. The eutectic means that the solute remain in equilibrium in frozen food. A frozen food held at -20^{o}C has an a_w of 0.823, irrespective of its composition. Although composition of food does not control a_w, it can have a marked influence on survival of microorganisms frozen in food. Sugars, sugar alcohol, glycols, and proteins, may have protective effect. The added sucrose have effect on the survival of *Torula* sp. in frozen orange juice.

7.8.2 Survival at Moderate Temperatures

Many sterile foods are microbiologically stable in the moderate or room temperature range. The majority of dried or concentrated foods, owe their stability to reduced level of a_w. However, if rehydrated before consumption, regain the ability to support microbial growth, so that the capacity of contaminating organisms to survive the period of low a_w storage is of obvious relevance. Studies on survival of pure cultures of vegetative bacteria equilibrated to range of a_w levels after freeze drying have shown clearly the increase in survival that accompanies reduction in a_w level to 0.1-0.2. In dried foods the bacteria during storage at reduced a_w levels is greatly influenced by the nature of solution from which they had been dried. While non-reducing sugars are protective, and reducing sugars accelerate bacterial inactivation.

7.8.3 Survival at Elevated Temperatures

Microorganisms vary in heat resistance, the more resistant bacteria (e.g. *Bacillus stearothermophillus*) producing spores with decimal reduction times in neutral foods as long as 4 minutes at 121°C. or 40 minutes at 110°C. Yeast ascospores are only slightly more heat resistant than vegetative yeast cells. The qualitative effect of moisture upon microbial heat resistant is well known-moist heat is a much more effective sterilizing agent than dry heat and, wherever, practicable, steam sterilization is preferred as being much more rapid than hot air (dry) sterilizing. Water activity is also likely to be significance in the heat treatment of foods in the intermediate moisture range, Pasteurization temperature for salmonellae, staphylococci, and yeast (50-60°C) death rates are lowest in the a_w range 0.75- 0.85 in glycerol adjusted solutions. Osmophilic yeasts respond similarly to salmonellae when heated in sucrose solution with decimal reduction times increasing as a_w decreases from 0.995 to 0.85. These organisms are more heat sensitive than salmonellae.

7.9 MICROBIAL SPOILAGE OF DRIED FOODS

Spoilage of dried fruits and vegetables by insects

The presence of water in fruits and vegetables is mandatory to undergo microbial spoilage. All organisms require water for carrying on their life processes. If the microorganisms cannot acquire the water it either dies or its further growth is arrested. Potential spoilage of a dried fruit, then depends upon how available water is to the spoilage microorganisms, It is therefore the thin demarcation line of water activity which establishes dehydration as a good preservative techniques. The degree to which water is available to the microorganisms is expressed by the term water activity, (a_w,) that is the vapour pressure of the solution divided by the vapour of the solvent.

Removing the water from the fruit and vegetables is to reduce its availability to the microorganisms. In a moist solid substance , the water vapour pressure is lower than the vapour pressure of free water at the same temperature because, in a solid substance, water reacts with polar group such as –CO -, -NH, -OH. Still further vapour pressure inside of capillaries (between plant cells) is lower than the vapour pressure of a plane surface of water. As the solutes present in the fruit are dissolved in water the vapour pressure is depressed. Certain osmophilic yeasts and certain xerophilic molds and fungi are able to live and proliferate at water activities of low values. These are the microorganisms responsible for the spoilage of dried fruit and vegetables. Bacterial growth of generally impossible when a_w is reduced below 0.90. The growth of normal yeast is generally impossible when the a_w is reduced below 0.88. The growth of normal molds is generally impossible below 0.80. Each organism has its own characteristics optimum a_w at which growth will occur. Molds are the most troublesome group of microorganisms will grow at a_w values below 0.70.

Dried fruits and vegetables are also subjected to insect attack when not dried and stored properly. Insect not only consume food stuffs but also leave much debris which spoils the appearance of the product. These insect can be killed either by heating or by fumigation. In heat treatment, dried fruits are dipped in boiling water or in dilute solution of salt (NaCl) –$NaHCO_3$) and then, redried at 54-65^oC. Dried vegetables may be heated directly without preliminary dipping. Fumigation with ethylene oxide inside the storage chamber also reduces attack by insects.

Dried fruits become musty or moldy and dried vegetables soft or slimy if kept in a damp atmosphere in unsealed containers. Hence, proper sealing and storing of containers at ambient temperature and in a dry place is important.

Dehydrated fruits and vegetable potential defects and means to prevent them are given below:

Defects	**Causes**	**Prevention**
Molding	High product moisture, above equilibrium relative humidity corresponding to water activity a_w = 0.70.	Reduce water content down to optimum values, pack in hermitic air tight package.
Infestation	Presence in dried products of larva or insects.	Storage room disinfection with toxic gases. Fumigation of packed products and of packages. Disinfection

		by heat (60-65^{o}C) of products before packing.

Considering the variety of natural food substances and the methods by which each is handled during processing , it is apparent that practically all kinds of microorganisms are potential contaminants. The type of food substance and the method by which it processed and preserved may favour contamination by certain groups of microorganisms. Most foodstuffs serve as good media for the growth of many different microorganisms, and microorganisms' changes in appearance, flavour odor, and other qualities of foods. These degradation processes may be descrived as follow;

Putrefaction

Protein foods + proteolytic microorganisms → amino acids + amines = ammonia + hydrogen sulphide.

Fermentation

Carbohydrate foods + carbohydrate-fermenting microorganisms → acids = alcohol = gases.

Rancidity

Fatty foods + lipolytic microorganisms→ fatty acids = glycerol.

Some microorganisms discolour foods as a result of pigment production. Slimes may be developed in or on foods by microorganisms capable of synthesizing certain polysaccharides.

Check Your Progress Exercise 6

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

1. At low moisture content how the growth of microorganisms is check.

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2. List the microorganisms which are responsible to spoil the dried fruits and vegetables.

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3. Write the level of a_w at which the growth of bacterial, yeast and mould is impossible.

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4. What are the changes occur in the food products when they are attack by microorganisms.

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7.10 LET US SUM UP

The reduction in weight and bulk by drying and dehydration of the commodity can result in economies in cost of containers, shipping and distribution of dehydrated products. The dehydration also result in the production of convenience products e.g. instant coffee instant milk and instant mashed potatoes. The production of dried fruits and vegetable products are less costly as there is a minimum of labour and processing equipment required. These products also require less space for storage then fresh canned or frozen fruits and vegetables.

Microorganisms are associated, in a variety of ways, with all of the food we eat. They may influence the quality, and availability of our food. Naturally occurring foods such as fruits and vegetables normally contain some microorganisms, and may be contaminated with additional organisms during handling. Food can serve as a medium for the growth of microorganisms, and this growth may cause the food to undergo decomposition and spoilage. The microorganisms on most of the dried fruits vary a few hundred per gram of fruits to thousands, and in whole fruits they are mostly on the outer surfaces. Spores of bacteria and molds are likely to be most numerous. When part of the fruit has supported growth and speculation of mold before or after drying, mold spores may be present in large numbers. Microbial counts on dried vegetables range from negligible to millions per gram. A number of genera of bacteria found on dried vegetables includes: *Escherichia, Enterobactor, bacillus, Clostridium, Micrococcus, Pseudomonas, Streptococcus, Lactobacillus* and *Leuconostoc*. Of these, *Lactobacillus* and *Leuconostoc* species are predominant in many samples of dehydrated vegetables.

Water activity (a_w) influences the physical, chemical and microbiological properties of many substances. The shelf life of foods, their colour, stability, taste, texture, vitamin content, aroma, mold formation and microbiological growth properties are influenced directly by the a_w value. The survival of microorganisms in dried foods can be markedly affected by a_w level, there are important interaction between a_w and such factors as pH, oxygen and food composition. Certain osmophilic yeasts and certain xerophilic molds and fungi are able to live and proliferate at water activities of low values. These microorganisms are responsible when water activity (a_w) is reduced below 0.88, and the growth of normal yeast is generally impossible below the water activity (a_w) of 0.88.

7.11 KEY WORDS

Water activity : Water activity (a_w) is the ratio of vapour pressure of food (P) and pure water (p_o) and expressed by $aw = p/p_o$.

Dehydration : Removal of moisture under controlled conditions of temperature, air flow and humidity.

Drying : Drying of the product under the source of non-conventional energy sources like sun and wind.

Blanching : Partial pre-treatment in which vegetables are heated in water or in steam to inactivate enzyme before processing.

Sulphuring : Exposing the fruits to the fumes of burning sulphure inside of closed chamber.

In-package desiccant : Packaging of the dried products with a material like calcium oxide or silica gel.

Sorption isotherms : Water sorption isotherms is a graphical presentation of data which shows the water relationship of food.

Preservation : Methods to hold food for a longer period than generally kept at ambient conditions. Food is safe, nutritive and free from and microbial infection.

ERH : Equilibrium Relative Humidity.

Osmotic dehydration : Removal of water through a membrane from higher concentration to lower concentration.

Sweating : Process for holding the dried fruits and vegetables in to bins or package for equalization of moisture

Pasteurization : Pasteurization of fruits and vegetable products by heat process below 100°C.

Rancidity : Discolouration of food products.

Processing : The application of heat to the fruit and vegetables after hermetic (air tight) sealing in containers is called the processing.

Spoilage : The food which has been damaged or injured which make the food undesirable for human use.

Rehydration ratio : Reconstitution ratio is the quantity of water replaced by dehydrated foods.

Reverse osmosis : Reverse osmosis means movement of water through the membrane by applying pressure on the solute side of the membrane in excesses of the osmotic pressure.

7.12 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   - Drying of commodity in the sun with non conventional sources of energy like sun and wind is called drying.
   - Drying the commodity under controlled conditions like temperature, relative humidity and air flow is called dehydration.

2. Your answer should include the following points:

   - To reduce the weight and bulk.
   - To reduce the water activity.

3. Your answer should include the following points:

    - To convey the heat to the product.
    - To let out the moisture from the product.

4. Your answer should include the following points:

    - They exhibits an energetic retention of moisture.
    - They bound the moisture to the solid content.

Check Your Progress Exercise 2

1. Your answer should include the following points:

    - Due to heat transfer to the product.
    - Due to mass transfer out the product.

2. Your answer should include the following points:

    - Heat transfer through conduction.
    - Heat transfer through convection.
    - Heat transfer through radiation.

3. Your answer should include the following points:

    - By addition of thermal energy to the product.
    - By transfer the heat to the product and water surface.

4. Your answer should include the following points:

    - Transfer of vapour from product surface to the air.
    - Transfer of heat from the air to the product.

Check Your Progress Exercise 3

1. Your answer should include the following points:

    - Dried fruits
    - Some types of toffee
    - Honey

2. Your answer should include the following points:

    - Osmophyile yeast
    - *Aspergillus echinulatus*
    - *Monascus bisporus*

Check Your Progress Exercise 4

1. Your answer should include the following points:

    - In the form of quality
    - In the form of availability
    - In the form of quantity

2. Your answer should include the following points:

    - By reduce the moisture content of the product.
    - By reduce the moisture content of their environment below critical level.

Check Your Progress Exercise 5

1. Your answer should include the following points:

    - Some of bacteria
    - Some of fungi
    - Some of viruses

2. Your answer should include the following points:

    - Load and types of microorganisms in raw material.
    - Time leg between preparation and drying.
    - Moisture content in the finished product.

3. Your answer should include the following points:

    - Bulbs should be free from diseases and blemish
    - Dried the cut slices immediately under control condition
    - Pack and stored under proper conditions

4. Your answer should include the following points:

    - Grading
    - Selection
    - Sorting

5. Your answer should include the following points:

    - Sweating
    - Pasteurization

Check Your Progress Exercise 6

1. Your answer should include the following points:

    - By arrest the growth of the microorganisms.
    - By destroys the microorganisms.

2 Your answer should include the following points:

    - Osmophilic yeast
    - Xerophilic molds
    - Fungi

3. Your answer should include the following points:

    - For bacterial growth a_w below, 0.90

- For yeast the a_w below , 0.88
- For mold growth the a_w below, 0.80

4. Your answer should include the following points:

   - Changes in appearance
   - Changes in flavour
   - Changes in odor
   - Changes in quality

7.13 SOME USEFUL BOOKS

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UNIT 8 CHEMICALS FOR CONTROLING MICROORGANISMS

Structure

8.0 OBJECTIVES

This unit introduces you to the concept of preservation of food with chemicals. After going through this unit you will be able to know how chemical reactions causing spoilage are prevented or delayed by use a wide range of chemical additives.

After studying this unit, you should be able to:

- know the various classes of chemical additives used in the food industry;
- explain how these chemicals help to prevent the spoilage of food;
- know the permitted and non permitted chemical additives;
- discuss the general considerations required in the selection of food preservatives; and
- that apart from certain added preservatives there are some naturally occurring preservative factors in food.

8.1 INTRODUCTION

In Unit 1, you read about the various types of microorganisms that are important in the food industry. In this unit, we shall tell you how the spoilage of food can be prevented or delayed which are caused due to these microorganisms or some other chemical reactions. This unit highlights the various classes of chemical preservatives that have been approved for the use

in food and their use. The various aspects to be considered for the selection of chemical additives (food additives), their mode of action and the adverse reactions resulting due to the consumption of the additives is also elaborated in this unit. The unit also deals with the developed additives, namely acids, alcohol and bacteriocins.

8.2 USES OF VARIOUS FOOD ADDITIVES AND CHEMICAL PRESERVATIVES

For centuries, man has recognized the effects of food additives and has used whatever was available-marigold for colour, wood ashes for leavening, the lining of calf stomachs for cheese making etc. today, thousands of compounds are used as food additives, whose chemical identity and structure are known. The use of food additives is imperative in the complex and integrated society in which we live. Additives have provided protection against food spoilage during storage, transportation, distribution or processing. Also, with the present degree of urbanization, it would be impossible to maintain food distribution without the processing and packing and packing with which many additives are involved.

Additives permit the variety of foods that we deem desirable and which certainly are objectively important in maintaining important nutrition. Vitamins and minerals are important in maintaining good nutrition. Many of these chemical additives can be manufactured so that foods can be "fortified" or "enriched".

There is then the need for the use of food additives to maintain the nutritional quality of food, to enhance the stability with resulting reduction in waste, to make food more attractive and to provide efficient aids in processing, packing and transport. The amount of food additives used should be kept to a minimum and it should conform to a standard of purity and be safe. Over 3000 different chemical compounds are used as food additives. They are categorized into different groups which will be discussed below.

According to WHO a food additive is defined as a substance or mixture of substances other than the basic foodstuff, which is present in food as a result of any aspect of production, processing, storage and packaging. The term does not include chance contaminants- thus the former refers to intentional food additive while latter is incidental un-intentional food additive.

Intentional food additives could be nutritive, freshness maintenance, sensory and processing aids; preservatives, antioxidants, emulsifiers, stabilizers, maturing agents, colours, special sweeteners, nutrient supplements, flavouring compounds and natural flavouring materials.

8.2.1 Types of Additives

- ***Acidity regulators***, used to alter and control the acidity or alkalinity levels for different desired effects, which can include preservation, added/altered tartness, colour retention and to assist raising agents.

- ***Acids***, used to control to what degree other substances function and/or to impart a sharp taste. Assists in the release of carbon dioxide in raising agents and can have a preservative effect.

- ***Anti-caking agents***, used to ensure the free flow in products such as dried milks, icing sugar and table salt.
- ***Anti-foaming agents***, used to reduce or prevent foaming (frothing) on boiling and to reduce scum forming.
- ***Antioxidants***, used to protect food against deterioration caused by exposure to air (oxidation), such as fat rancidity, flavour deterioration or colour changes.
- ***Bleaching agents***, used to artificially whiten flour.
- ***Buffers***, see acidity regulators.
- ***Bulking agents***, used to increase volume without significantly adding to the energy levels of the food. Normally used in diet foods but can also be used to pad out expensive ingredients. Not usually digested and acts as a source of dietary fibre (roughage).
- ***Carriers and carrier solvents***, used to modify a food additive (by dissolving, diluting or dispersing etc.), without changing its function, to enable easier use or handling.
- ***Emulsifiers***, used to aid in the formation and maintenance of the dispersion of two or more substances which would normally separate and not normally mix, such as oil and water. Milk, mayonnaise and salad dressings are typical oil in water emulsions, butter and margarine water in oil emulsions.
- *E*mulsifying salts, used to disperse protein so reducing the stringiness in cooked cheese.
- ***Firming agents***, used to make or retain firmness or crispness in fruit and vegetables and to strengthen gels.
- ***Flour improvers***, used to enhance the elastic properties and aid the development of dough. Also accelerates the effect of bleaching agents.
- ***Foaming agents***, used to provide a uniform dispersion of gas in a food.
- ***Gelling agents***, used to form a jelly so providing texture to a product.
- ***Glazing agents***, used to produce a protective coating or to impart a polish/sheen on the surface of a food such as confectionery or citrus fruit.
- ***Humectants***, used to retain moisture in foods by absorbing water from the air to prevent drying out.
- ***Modified starch***, used for various functions including adding texture, adding bulk, stabilizing and as a thickener.
- ***Packaging gases***, used to replace air in the packaging of foodstuffs susceptible to oxidation but not necessarily shown on food labels.

- ***Preservatives***, used to extend the shelf-life of products by preventing the growth of microorganisms which could otherwise cause food decay and, in some cases, food poisoning.

- ***Propellants***, a gas or volatile liquid used to expel foodstuffs from aerosols.

- ***Raising agents***, used to increase the volume of doughs and batters by promoting gas release (aeration).

- ***Releasing agents***, used to prevent foodstuffs sticking to machinery, molds, packaging etc. but not necessarily shown on food labels even though some may remain in the food.

- ***Sequestrants***, used to combine with trace metals in the environment to render them inactive.

- ***Stabilizers***, used to maintain the physical state of a food and to stabilize, retain or intensify the existing colour of a food, particularly emulsions, and therefore often used with emulsifiers.

- ***Sweeteners***, there are two different types of sweeteners:

    1. **Intense sweeteners** – these have a sweetness many times that of sugar and are therefore used at very low levels. They are used in products such as diet foods, soft drinks and table top sweeteners;

    2. **Bulk sweeteners** – these have a similar sweetness to sugar and are used at comparable levels. Unlike intense sweeteners they also provide bulk (although their main function is to provide sweetness). They are used in products such as sugar-free confectionery and foods for diabetics.

- ***Thickeners***, used to increase viscosity, modify texture and impart stability.

8.2.2 Role of Food Additives

Food additives help to enhance the consumer acceptability, help in maintaining or improving the nutritional quality, enhance stability or keeping quality by acting as antimicrobial agents with the resulting reduction in waste and prevention of chemical and biological deterioration, make food more attractive and provide sufficient aids in the food products for improving texture, colour and flavour, check spoilage by inactivating microorganisms and maintain safety of foods, facilitate preparation and help to improve palatability of the product.

It helps to enhance the shelf life of food or food products. It has been estimated that we consume about 5 kilograms of food additives as preservatives, colours, bleaches, flavours, emulsifiers and stabilizers every year in the food we eat. This not only results in extra work for our body to remove them, but frequently trigger asthma attacks; rashes; respiratory disturbances; hyperactivity in children, and in some people, an abnormal sensitivity to prescribed medications, particularly aspirin. Below are some common additives found in refined foods, and well-worth avoiding by those susceptible to their effects.

Acceptable daily intake (ADI) for various preservatives

Preservative	ADI (mg/kg body wt/day)
Acetic acid including its Na/K salts	No limit
Sodium diacetate	0-15
Benzoic acid including its Na/K salts	0-5
Formic acid	0-3
Hexamethylene tetramine	0-0.15
Para hydroxy benzoic acid esters	0-10
Lactic acid and its salts	No limit
Propionic acid and its salts	No limit
Natamycin/pimaricin	0-0.3
Na NO_3 and KNO_3	0-5
$NaNO_2$ and KNO_2	0-0.2
Sorbic acid including its Na/K/Ca salts	0-2.5
SO_2, Na_2 SO_3, $NaHSO_3$, Na/K metabisulphite	0-0.7

8.2.3 Preservatives

Preservatives are substances which are capable of inhibiting, retarding or arresting the process of fermentation, acidification or other decomposition of food or of masking any of the evidence of putrefaction but it does not include salt, sugar, vinegar, glycerol, alcohol, spices, essential oils etc. Sulphur dioxide (including sulphites) and benzoic acid (including benzoates) are among the principle preservatives used in the food processing industry. The permitted quanitity of sulphur dioxide and benzoic acid is given in the following tables.

Food additives and their usage concentrations

Food additives	Concentration (%)	Foods
Antioxidant : Butylated Hydroxy Anisole (BHA)	Not exceeding 0.02% of the total fat content and 0.01% of the finished product 0.02	*Rasogolla* and *Vadas* Whole and partially skimmed milk powder Margarine
Colours	0.02	Most foods
Flavour : Monosodium glutamate	0.05	Meat product, soup powder
Anticaking agent: Aluminum silicate	2	Table salt, onion powder, garlic powder, soup powder
Sweetening agent : Saccharin	100 ppm	Carbonated non-alcoholic drinks
Sequestrant : Ethylene Diethyle Tetra Amino Acetic Acid (EDTA)	33-800 ppm	Canned carbonated beverages, salad dressings and margarine

Classes of preservatives

CLASS I:

Common salt, sugar, dextrose, spices, vinegar or acetic acid, honey

CLASS II:

Benzoic acid and its salts, sulphur dioxide and the salts of sulphurous acid, nitrites and nitrates, sorbic acid and its salts, propionic acid and its salts, lactic acid and its salts.

Sulphur dioxide

Sulphur dioxide and its derivatives have been extensively used in foods as a food preservative. It acts both as an antioxidant and reducing agent and prevents enzymatic and non-enzymatic reactions, leading to microbial stability. The common used forms are sulphur dioxide gas and sodium, potassium and calcium salts of sulphite, bisulphite or metabisulphite. It is like a biocidal and biostatic agent and is more active against bacteria than molds and yeasts.

Sulphite or metabisulphite sprays or dip with or without added citric acid provides effective control of enzymic browning in pre-peeled and pre-sliced potatoes, carrots, mushroom and apples.

Sodium benzoate

It was the first chemical preservative permitted in foods by the FDA, and it continues in wide use today in a large number of foods. Benzoates have greatest activity at low pH. As used in acidic foods, benzoates act essentially as a mold and yeast inhibitor.

In foods such as fruit juices, benzoates may impart disagreeable tastes at the maximum level of 0.1 per cent. The taste has been described as being ‘peppery ‘or burning.

Permitted quantity of benzoic acid in food

Processed food	Permitted quantity of Benzoic Acid (ppm)
Non-alcoholic wines, squashes, crushes, fruit syrups, cordials, fruit juices and barley water (to be used after dilution)	600
Jams, marmalades, preserves, canned cherry, fruit jelly	200
Sweetened mineral water and sweetened ready to serve beverages	120
Brewed ginger beer	120
Pickles and chutneys	250
Tomato and other sauces	750
Danish tinned caviar	50
Tomato puree and paste	750
Syrups and sherbets	600
Fat spread	1000

Quantity of sulphur dioxide permitted in food

Processed food	Permitted quantity of SO_2 (ppm)
Sausages and sausage meat containing raw meat, cereals and condiments	450
Fruit, fruit pulp or juice (not dried) for conversion into jams or crystallized glace or cured fruit or other products	
a) cherries	2000
b) straw berries and raspberries	2000
c) other fruits	1000
Fruit juice concentrate	1000
Dried fruits	
Apricots, peaches, apples, pears and other fruit	2000
Raisins and sultanas	750
Other non-alcoholic wines. squashes, crushes, fruit syrups, cordials, fruit juices and barley water (to be used after dilution)	350
Jams, marmalade, preserves, canned cherry and fruit jelly	40
Crystallized glaced or cured fruit (including candied peel)	150
Fruit and fruit pulp not otherwise specified in the schedule	350
Plantation white sugar, cube sugar, dextrose, *gur*, jaggery or *misri*	70
Khandsari(s) and *Bura*	150
Refined sugar	40
Corn flour and similar starches	100
Corn syrup	450
Canned *rossogullas*	100
Gelatin	1000
Beer	70
Cider	200
Alcoholic wines	450
Sweetened mineral water/ready to serve beverages	70
Pickles and chutneys made from fruits or vegetables	100
Dehydrated vegetables	2000
Syrups and sherbets	350
Dried ginger	2000
Hard boiled sugar confectionery	350
Dry mixes of *rossogullas*	100

8.2.4 Acidulants

Sour or acidic taste of a food is attributed to the acidic components present in the food. Many processed foods and beverages, however, need the addition of acids to impart characteristic taste and flavour to the final food product. The intensity of sourness and ability to reduce pH vary among the organic group of acidulants in the decreasing order as follows:

Fumaric > tartaric > malic > acetic > citric > lactic > gluconic acid

Commonly used acidulants include acetic, adipic, citric, fumaric, lactic, malic, phosphoric and tartaric acids. Citric acid is the most versatile and widely used food acidulant.

Main foods in which acidulants occur or added to food

Acid	**Main food**
Acetic acid	Pickles, sauces, relishes, fermented vegetables and fruits, vinegar, wheat bread, cheeses and creams, apple juice, grapefruit juice.
Adipic acid	Beet juice, guava, papaya, raspberry, pork fat, dairy foods, gelatin and desserts, puddings, beverages, jams and jellies, snack foods, condiments
Citric acid	Oranges, lemons, grapefruit, black currants, gooseberries, pineapple, raspberries, strawberries
Fumaric acid	Confectionery, powdered gelatins, desserts, cheese cake, jams, and jellies
Glucono-delta-lactone	Cured meats, frankfurters, salami, sausages, dessert mixes, bakery mixes, processed cheese, fish products, spice preparation
Lactic acid	Fresh meat, yogurt, cheese, bread, pickles, sauces, relishes, fermented foods, buttermilk, wines, beer
Malic acid	Watermelon, plum, apple, cherry, peach, pear, grape, gooseberry, pineapple
Phosphoric acid	Cola beverages, jams and jellies, bread dough, cake, flour
Tartaric acid	Grapes, tamarind, pineapple, mulberries, gherkins, wines

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 additives. What are intentional and unintentional food additives?

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2. Briefly discuss the functions of food additives.

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8.2.5 Control of Psychotropic Contamination in Food

Increasingly all types of consumers are demanding minimally processed foods that are high in quality, nutritionally superior and easy to prepare. Food processors have met this demand by developing refrigerated foods with extended shelf life. The most important bacteriological problem in processed food products today is deterioration due to contamination by psychotropic microbes, as storage at low temperature is favourable for the growth of high levels of psychrotrophic microorganisms. Psychotrophs are bacteria, yeasts and molds that grow although slowly, at refrigeration temperature (below 7°C) but grows optimally at temperatures above refrigeration, e.g. 25-30°C. Their maximum growth temperature is 30-35^{o}C. Several pathogens such as Aeromonas hydrophila, Clostridium botulinum, Listeria spp., Yersinia enterocolitica, some strains of Bacillus cereus, enteropathogenic Escherichia coli and Vibrio parahaemolyticus can grow at refrigeration temperature. These bacteria may enrich in food during cold if storage times are long enough. Some of these pathogens can cause illness when even few cells are ingested.

The control of psychrotrophs in food products should start from the very beginning of raw material procurement up to processing and storage of the finished product. The following steps are of considerable importance. Processors need to select high-quality raw materials with low levels of microorganisms, especially psychrotrophs. Fabrication of raw materials by using clean and sanitized machinery and equipments into finished products

under hygienic conditions is also important. Apart from this regular check on quality of water supply and proper chlorination of water used in the food industry should be done. Appropriate use of salt and other ingredients which reduces the a_w to 0.98 or below will lengthen the lag phase of most bacteria and will further reduce the rate of any subsequent growth. Most recent studies have shifted to the use of lactic acid bacteria that produces bactericidal chemicals called bacteriocins to slow or inhibit the growth of psychrotrophic organisms. By lactic acid bacteria a wide variety of food borne pathogens are either inhibited or killed, and many spoilage organisms are affected in similar ways, especially Gram-negative psychrotophs.

Thus the effective control of microbial contaminants must begin on the farm and be followed through to the retail store. Clean equipment and packages, use of approved food additives and chemical preservatives of Generally Recognized As Safe (GRAS) status in proper concentration, limited time of storage, low holding temperatures for raw materials and the finished product, effective laboratory control and attention to good manufacturing practices which will slow the outgrow of psychrotrophs will help the food plant to produce with good yield, good flavour, long shelf –life and high sales appeal.

8.3 GENERAL CONSIDERATIONS IN THE SELECTION OF CHEMICAL FOOD PRESERVATIVES

Antimicrobial preservatives added to foods can be grouped as follows:

1. *Those added preservatives not defined as such by law:* natural organic acids (lactic, malic, citric etc.) and their salts, vinegar, sodium chloride, sugars, spices and their oils, woodsmoke, carbon dioxide and nitrogen
2. *Substances generally recognized as safe (GRAS) for addition to foods:* propionic acid and sodium and calcium propionate, caprylic acid, sorbic acid and its salts, sulphur dioxide, sodium nitrite
3. *Chemicals considered to be food additives*, which would include all that are not included in the first two categories
4. *Chemicals proved safe and approved by the Food and Drug Administration*

8.3.1 Desirable Properties of Food Preservatives

There are seven requirements for food preservatives:

- No toxicity problems.
- Microbiocidal rather than microbiostatic properties.
- Must be stable in foods (especially if only microbiostatic).
- The spectrum of activity should correspond to the spectrum of micro-organisms likely to appear in the food.
- Must not stimulate the development of resistant strains of microorganisms.
- Chemicals used therapeutically are not recommended as food additives.
- An assay procedure should be available.

Categories of Antimicrobial Food Additives Added to the Food:

Following chemicals and biochemicals are used in food preservation:

- Naturally present or formed in the food, chemicals added to the food, bacteriocins e.g. lactoperoxidase enzyme, lysozyme, lactoferrin, nisin etc.
- Chemicals with antimicrobial properties of salts of organic acids, like citric, benzoic, propionic and ascorbic. Chemical preservatives (sulphur dioxide and sulphites, parabens etc.), nitrites and nitrates.
- Chemicals with multifunctional properties added to the food, one property being antimicrobial e.g. spices and essential oils, salt, sugar, antioxidants, vinegar etc.

8.3.2 Mode of Action of Food Additives

- Alteration of cell wall permeability.
- Alteration of colloidal nature of protoplasm.
- Damage of the cell wall.
- Damage of proteins.
- Inhibition of enzyme activity.
- Disruption of cytoplasmic membrane.
- Bacteriostatic or bactericidal action (toxicity of the antimicrobial agent towards microorganisms).
- Interference with synthetic processes.

8.3.3 Factors Affecting the Antimicrobial Activity of Food Additives

Many factors must be considered for the selection of a specific antimicrobial food additive for a specific food. These factors are as follows:

- Physical and chemical properties of the antimicrobial agents (such as water solubility, hydrophobic lipophyllic balance, boiling point, ability to ionize and potential interaction with food constituents). The activity of the antimicrobial is reduced as a result of reaction with lipids, proteins or carbohydrates.
- Composition of food, its pH/ acidity and nutritional value.
- Type of preservation system other than chemicals used in the food.
- Characteristics and number of microorganisms.
- Initial contamination by microbes prior to preservation/processing.
- Type and concentration of chemical used.
- Time and temperature of food storage.
- Cost and toxicity of the antimicrobial.

8.3.4 Precautions to be taken for Using Food Additives

- Food additives must be thoroughly tested before use. Foods containing physical hazards such as stones, seeds, glass fragments or metal pieces must be thoroughly checked.
- FDA approved food additives should be used. Improper use of some of them may prove to be harmful to human health.

- Use of additives should not be permitted if:
  - They fail to serve the interest of consumers.
  - They are used to mark the effect of faulty processing and handling techniques.
  - They are used to deceive the consumers.
  - Their use results in a significant reduction in the nutritive value of the foods.
  - Additives should be used in a controlled way so as to maximize benefits and prevent abuses.

Status of some additives and acceptable dietary intake (ADI)

Material	**Status**
Amaranth (red ozo dye)	Carcinogenic, but still WHO/FAO-prescribed an ADI* for Amaranth
Saccharin	Bladder cancer. However, still permitted in soft drinks to the extent of 100ppm. WHO/FAO-prescribed ADI from 2.5-5.0 mg/kg of body weight.
Cyclamate	Bladder cancer. WHO/FAO-prescribed ADI of 4 mg/kg of body weight for cyclamate.
Brominated vegetable oil	Banned in India and UK
Hydrogen peroxide	Used for extending shelf-life of milk, is repeatedly turned down on grounds that it would have undesirable consequences on milk collection practices.
Gallate, Phenols	Permitted in most countries, but in India there use required specific permission which is not granted.
Nitrates and nitrites	Give rise to nitrosamine which are carcinogenic but still used in our country within the permissible limit.
Sulphites	Banned in USA

*ADI-Acceptable Daily Intake (mg/kg body weight/day)

8.3.5 Adverse Effects of Using Food Additives

Although these additives are regarded as GRAS (generally recognized as safe), their increased used may also lead to various health problems viz. acidity, dyspepsia, digestive disorders etc.

Food additive name	Often used in	Common reactions
Tartrazine (colour)	drinks, cakes, snacks, ice-cream, confectionery	asthma; hyperactivity; aspirin sensitivity
Sunset yellow (colour)	drinks, packet soups, dessert, biscuits, confectionery, ice-cream	hyperactivity; allergies; aspirin sensitivity
Cochineal (colour)	cakes, confectionery, ice-cream	hyperactivity
Azorubine (colour)	packet soups, sauces, jams, desserts (jellies)	asthma; hyperactivity; asprin sensitivity
Indigotine (colour)	tablets, capsules, ice cream, biscuits	nausea; skin rashes; allergies; high blood pressure
Brilliant blue (colour)	tinned peas, bacon-flavored snacks	hyperactivity
Caramel (colour)	drinks, sauces, soups, cakes, pickles, vinegar	hyperactivity
Benzoic acid (preservative)	confectionery, cheeses,	asthma; hyperactivity;
Sulphur dioxide (preservative)	beer, wine, soft drinks, dried fruit, cordials	asthma; hyperactivity
Sodium bisulphite (preservative)	wine, beer, soft drinks, juices, cordials	asthma; destroys vitamin B1; hyperactivity
Sodium nitrite (preservative)	cured meats, some cheeses	hyperactivity; adverse reactions in children; potentially carcinogenic
Propyl gallate (antioxidant)	oils, margarine, salad dressings	gastric and skin irritant
Tert-butyl hydroquinone (antioxidant)	fats, oils, margarine, packet chips	nausea; delirium
Butylated hydroxyanisole (antioxidant)	fried snacks, soft drinks, edible oils, margarine, chewing gum	hyperactivity; asthma; adverse reactions; allergies; increases cholesterol levels
Carageenan (thickener) (emulsifier)	ice-cream, jellies, cake decorations, cheese, salad dressings	allergies; intolerances
Mannitol (emulsifier)	icecream, confectionery, low calorie foods	allergies; diarrhoea, nausea
Monosodium glutamate (MSG) (flavour enhancer)	prepacked meals, snacks, Chinese cooking	hyperactivity; asthma; adverse reactions; allergies; aspirin sensitivity

Disodium 5' ribonucleotide (flavour enhancer)	flavoured crisps, instant noodles, party pies	skin rashes; not easily broken down by body
Aspartame (sweetener)	diet drinks, diabetic confectionery, ice cream	allergies; headaches; nervous disorders

8.4 DEVELOPED AND ADDED PRESERVATIVES

Developed preservatives include those synthesized naturally, by various microorganisms during fermentation and growth and metabolism.

8.4.1 Acids Produced during Fermentation

Food fermentations may serve either or both of two purposes: (1) to produce new and desired flavours and physical characteristics and hence a different food product and (2) to help preserve the food. The preservatives produced in foods by microbial action are the most part acids (chiefly lactic) and alcohol. The preservative effect of these substances nearly always is supplemented by one or more additional preservative agents, such as low temperature, heat, anaerobic conditions, sodium chloride, sugar or added acid.

Developed acidity plays an important part in the preservation of sauerkraut, pickles, green olives, fermented milk, cheese and certain sausages and in various fermented foods of plant origin. Development of full amount of acidity from the sugar available may be permitted in the pickle and green olive fermentations, or the fermentation may be stopped by chilling or canning before the maximum acidity is attained in other fermentations. The approximate acidity developed in some of these products, expressed as lactic acid, is sauerkraut, 1.7 per cent: dill pickles and green olives, 0.9 per cent and fermented milks, 0.6 to 0.85 per cent.

8.4.2 Alcohol

The alcohol content of beer, ale, fermented fruit juices and distilled liquors has a preservative effect but was not produced primarily for that purpose.

8.4.3 Bacteriocins

Many natural products have been found to have efficient preservative effect and their application in food is catching up fast due to the increased awareness about their nutritional and health benefits. These are termed as bio-preservatives as they act on harmful spoilage and pathogenic microbes and prevent their growth in foods.

Bacteriocins constitute an important segment of these biopreservatives. Technically speaking, the bacteriocins are proteinaceous antimicrobial compounds that kill or inhibit closely related bacteria and also are capable of exhibiting a wide inhibitory spectrum against spoilage and pathogenic bacteria. Various microorganisms such as the lactic acid bacteria (comprising species of *Lactobacillus, Lactococcus, Leuconostoc* and *Pediococcus*) and species of *Corynebacterium, Propionibacterium, Enterococcus, Bacillus* and *Escherichia* have been reported to produce bacteriocins or bacteriocin-like inhibitory substances. Lactic acid bacteria have been shown to produce sufficient quantities of bacteriocins in various cultured and fermented food preparations

(*dahi*, yogurt, cheese etc.) to prevent the growth of harmful bacteria. Alternatively the purified bacteriocin preparation can also be added directly to the food. So far, researchers have extensively tried out only two bacteriocins, namely nisin and pediocin as biopreservatives in various food systems of which nisin is the only bacteriocin that has been approved as a GRAS food additives.

Nisin is a well known and most widely used bacteriocin produced by *Lactococcus lactis* subsp. *lactis* (formerly *Streptococcus lactis*). It has been used in processed cheese, pasteurized milks, flavored milk and various other dairy products, in addition to canned foods and alcoholic beverages. The recommended doses of nisin used varies from 100-150 IU/g depending on the type of food. Nisin has sporostatic activity. This results in significant energy savings in canning processes by way of low heat application. So it is useful for the non-thermal preservation of foods. Nisin also has a great potential for use in brewing industry. It also finds application in low pH foods. In many European countries nisin has affirmed GRAS status in 1998 by Food and Drug Administration (FDA) for use as an antimicrobial agent.

Besides nisin, several other bacteriocins produced by lactic acid bacteria include pediocin PA-I and pediocin AcH produced by Pediococcus *acidilacti*, sakacin A from *Lactobacillus sake*, plantaricin from *Lactobacillus plantarum*, acidophilicin LA-I from *Lactobacillus acidophilus* and helveticin J produced by *Lactobacillus helveticus* and so on. The Pediococci which are used as starter cultures in certain vegetable and meat fermentations have also been the subject of recent investigation with regard to their bacteriocin-producing ability.

Advantages of using bacteriocins: The bacteriocins offer several advantages over the preservatives that are presently being used in several foods. They do not have any ill effect on the health of the consumer so they are safe to use and the inhibitory effect of bacteriocins on the growth of microorganisms exhibits the potential to inactivate microorganisms in foods.

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. Define bacteriocins. Give a few examples and possible uses.

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2. What are the desirable characteristics of food additives?

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3. List down the mode of action of food additives.

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8.5 LET US SUM UP

Chemical additives that control microorganisms are not only a technological and functional tool in hands of food technologists but also aid in restoring the nutrients lost through processing. The availability of chemical/food additives has allowed the production of numerous out-of-season foods and a variety of new food products. Additives have increased the development of convenience foods, snack foods, low-calorie and health promoting (functional) foods, exotic foods and a variety of food substitutes. The present day consumer demands high quality, convenient and minimally processed foods. Some products can be formulated with ingredients such as organic acids, chemical preservatives, nitrite, bacteriocins, high concentrations of salt, carbon dioxide etc. that are barriers to microbial growth and can also inhibit there growth due to their antibacterial and antifungal properties. The role of chemical additives thus, becomes all the more important, hence to be selected judiciously, keeping in view their toxicological and biochemical role in food, before they are recommended and they have become an integral part of food industry for day to day life for the production of various processed products. They help to assure a food supply with the safety, variety, appeal, wholesomeness and affordability we have become accustomed to.

8.6 KEY WORDS

Food Additive : Food additive is defined as a substance or mixture of substances other than the basic foodstuff, which is present in food as a result of any aspect of production, processing, storage and packaging. The term does not include chance contaminants.

Bacteriocin : Proteinaceous antimicrobial compounds that kill or inhibit closely related bacteria and also are capable of exhibiting a wide inhibitory spectrum against spoilage and pathogenic bacteria.

Nisin : Widely used bacteriocin produced by *Lactococcus lactis* subsp. *lactis* (formerly *Streptococcus lactis*).

GRAS : Substances generally recognized as safe

ADI : The Acceptable Daily Intake (ADI) is defined as an estimate of the amount of a food additive, expressed on a bodyweight basis that can be

ingested on a daily basis in the diet over a lifetime without appreciable risk to health. "Without appreciable risk" means the practical, in view of the actual level of knowledge, certainty that no harm will result, even after a lifetime of exposure to the chemical additive concerned. The ADI is usually given as a range of 0-x milligrams per kilogram of bodyweight per day.

8.7 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1.
   - Food additive is a substance or mixture of substances other than the basic foodstuff, which is present in food as a result of any aspect of production, processing, storage and packaging.
   - Intentional food additives are added deliberately to food and could be nutritive, freshness maintaining, sensory and processing aids; preservatives, antioxidants, emulsifiers, stabilizers, maturing agents, colours, special sweeteners, nutrient supplements, flavouring compounds and natural flavouring materials.
   - Unintentional food additives are chance contaminants which may get incorporated into food during any step of processing and are not desirable.

2. Food additives help to:
   - Enhance consumer acceptability.
   - Help improve or maintain the nutritional quality.
   - Enhance stability and prevent deterioration.
   - Make food more attractive and palatable.
   - Maintain the safety of foods.

Check Your Progress Exercise 2

1.
   - Bacteriocins are proteinaceous antimicrobial compounds produced by bacteria, that kill or inhibit closely related spoilage and pathogenic bacteria eg: Nisin, Pediocin, Acidophillin etc.
   - They are used in processed cheese, pasteurized milks, flavored milks and various other dairy products, canned foods and alcoholic drinks, brewing industry etc.

2. Food additives should be non-toxic, economical, must be stable in foods, should be microbiocidal rather than microbiostatic, should have broad antimicrobial spectrum, must prevent growth of resistant strains and an assay procedure should be available to detect them.

3. Action of food additives is by:
    - Altering cell wall permeability of bacteria.
    - Altering its protoplasm.
    - Damaging proteins and cell wall.
    - Inhibition of enzyme activity of cell.
    - Disruption of cell membrane and interfering with cell synthesis processes.

8.8 SOME USEFUL BOOKS

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UNIT 10 FOOD INTOXICATIONS

Structure

10.0 OBJECTIVES

After reading this unit, you will be able to:

- discuss the causal organisms responsible for food borne intoxications;
- explain the toxins production by mold and bacteria (*Clostridium botulinum* and *Staphylococcus aureus*);
- the microbial toxins produced, the foods associated in intoxication, symptoms of the disease, diagnosis, conditions necessary for outbreak and preventive measures required will also be discussed; and
- know the naturally present toxins in the food products will also be accounted for.

After reading this unit you will be able to distinguish between food borne intoxications caused by the various microbiological agents and their preventive measures.

10.1 INTRODUCTION

We have already studied about the food borne diseases and their classification. Food borne intoxications are basically food borne illness caused due to ingestion of toxin produced my microorganisms (mycotoxins, bacterial toxins). Natural toxins present in food may also result in food poisoning in humans.

Food poisoning is also caused by consuming old, used, residual, fermented, spoiled, contaminated, toxic and bacteria infested food.

10.2 NATURAL TOXINS

Some plants and animals originate food contain toxic substances. Some pulses and legumes contain a number of toxic substances such as protease inhibitors, lathyrogens, and flavism causing agents, cyanogens, haemaglutinins and paponins which are discussed below:

a) Trypsin inhibitor is a proteinous in nature. It suppresses the release of amino acid. It thus interferes with the normal growth of animals fed with such pulses.

b) Haemglutinins are also proteins. They impair the absorption system.

c) Cynogenic glycosides cause cyanide poisoning on hydrolysis of the glycoside by the enzyme α-glucosidase, hydrogen cyanide is liberated. A cyanides content of 10-20 mg per 10 gm of pulses is considered safe. Many legumes excepting limabean (*Phaseolus lunatus*) contains cyanide within these limits.

d) Saponins are glycosides of high molecular weight. This has been reported in soyabean, swordbean and jackbean. Toxic saponins cause nausea and vomiting and can be removed by soaking the beans prior to cooking.

e) Alkaloids are known to occur in the seeds of many legumes but they are relatively innocuous.

f) Some compounds present in pulses appear to bind iodine thus producing a state of iodine deficiency in the thyroid and eventually goitre.

g) Lathyrism is a disease that paralyses the lower limbs. The disease is associated with consumption of *kesari dal* regularly as high as 300g daily. In lathyrism, the toxic substances interfere with formation of normal collagen fibers in the connective tissue.

h) A hemolytic factor in *Vicia faba* causes flavism. It is caused by eating broadbeans or by inhaling pollen of its flowers. Flavism is hemolytic anemia. In several cases, death may occur within 24-48 hours of the onset of the attack.

i) Oxalic acid, a constituent of rhubarb, spinach and beet may cause oxalic poisoning in certain individuals.

j) Some poisonous substances may also be present in some cereals and vegetables e.g. protease inhibitor in cereals and potatoes, saponins in spinach and asparagus and goitrogens in rapeseed mustard, cabbage and related species. Goitrogens cause hypothyroidism and thyroid enlargement

k) Tissues of certain marine animals contain toxic substances, which cause adverse responses when eaten. Some algae like *Gymnodinium* and *Gonyaulax* are toxic. Heating does not destroy these substances.

l) Algal or Planktonic Fish Poisonings: Fish poisoning can result from the ingestion of fish or shellfish that have fed upon algae toxic to human beings. Paralytic shellfish poisoning is caused by ingestion of shellfish such as scallops, clams and mussels which have consumed toxic dinoflagellates. Symptoms appear within 10min after ingestion and include gastrointestinal distress, parasthesia of the lips and fingertips followed by ataxia, muscular uncoordination and ascending paralysis. Death may occur within 2 to 12 hours from cardiovascular collapse or respiratory failure. The human lethal dose of toxin is considered to be 3 to 4 mg.

Prevention: Soaking, heating or fermentation of pulses can reduce or eliminate most of the toxic factors in them. Heat causes denaturation of the proteins responsible for trypsin inhibition and haemagglutination and of the enzyme causing hydrolysis of cyanogenic glycosides. Fermentation also destroys toxic factors and yield more digestible products of high nutritive value.

10.3 MYCOTOXINS

Fungi are a very diverse group of organisms and have a significant impact on the production, spoilage and safety of food. Molds have not only served to synthesize antibiotics but also to produce some foods. Fermented foods such as some cheese, soy sauce, *miso, tempeh* and other oriental delicacies are prepared with the help of molds.

It is well documented that some molds produce toxic substances. Some fungi elaborate the toxin in large macroscopic fruiting bodies; for example, the toxin produced by certain species of *Amanita*, a poisonous mushroom. Other fungi always grow and sporulate as parasites on living host plants, and sometimes will do so only on a specific host. *Claviceps* is an example of this group of fungi and it produces mycotoxins. In contrast to fungi that are parasitic on living plants another group of fungi is saprophytic and causes destruction of dead plants and animal material. There is abundance of the spores of these molds in atmosphere and are found to inhabit stored grain and dried products and hence have been referred to as "storage fungi". These molds include *Cladosporium, Fusarium, Penicillium, Aspergillus* and *Alternaria*.

Mycotoxins are secondary metabolites produced by molds on foodstuffs that causes illness or death when ingested by man or animals. The primary metabolites are those that are essential for growth whereas secondary metabolites are formed during the end of the exponential growth phase and have no apparent significance to the producing organism relative to growth. The mycotoxins commonly encountered in food are around one million times less toxic than most lethal of the botulism toxin. But long term chronic toxicity is of special concern because several of the mold metabolites are carcinogenic and influence the immune response of a number of animal species. The syndrome resulting from ingestion of toxin in a mold contaminated food is referred to as mycotoxicosis.

At the beginning of the last century, two major mycotoxicosis caused considerable suffering and mortality. They were alimentary toxic aleukia (ATA) in Russia, caused by consumption of corn contaminated with T-2 toxin produced by *Fusarium sporotrichoides* and yellow rice disease in Japan, associated with *Penicillium islandicum*. More recently, outbreaks of aflatoxicosis caused by consumption of corn contaminated with *Aspergillus flavus* were reported from India involving approximately 1000 people of whom nearly 100 died.

Several very important mycotoxins such as the sporidesmins, slaframine and tremorgens are associated with animal feeds and forages which affect the quality of meat and other animal products. It is also seen that mycotoxins present in animal feed get into human foods because they pass through the food chain in either their original or metabolized form.

When we store foods under inappropriate conditions they are susceptible to mold growth. Many mycotoxigenic species are able to produce several mycotoxins. It is likely, therefore, that contaminated foods will contain a cocktail of toxins that can interact synergistically.

Some major mycotoxins found in foods

Mycotoxin	Major Foods	Common Producing Species
Aflatoxins	Corn, groundnuts, figs, tree nuts	*Aspergillus flavus, A. parasiticus*
Aflatoxin M_1	Milk, milk products	(secreted by cow after metabolism of Aflatoxin B_1
Deoxynivalenol	Cereals	*Fusarium graminearum, F. culmorum*
Fumonisin	Corn	*Fusarium moniliforme*
Ochratoxin	Corn, cereals, coffee beans	*Penicillium verrucosum, Aspergillus ochraceus*
Patulin	Apple juice	*Penicillium expansum*
Sterigmatocystin	Cereals, coffee, beans, cheese	*Aspergillus versicolor*
Zearalenone	Corn, barley, wheat	*Fusarium graminearum*

10.3.1 Aflatoxin

Aflatoxins are the most widely studied of all mycotoxins. Knowledge of their existence dates from 1960, when more than 100,000 turkey died in England after eating peanut meal imported from Africa and South America. From the poisonous feed were isolated *Aspergillus flavus* and a toxin produced by this organism that was designated aflatoxin (*Aspergillus flavus* toxin- A-fla-toxin). These compounds are highly substituted coumarins, and at least 18 closely related toxins are known. Aflatoxin B_1 is the most important of this large family of compounds and is produced by *Aspergillus flavus, A. parasiticus* and *A.nominus*. The toxicity of the six most potent aflatoxins decreases in the following order: $B_1>M_1>G_1>B_2>M_2\neq G_2$.

Occurrence: Aflatoxigenic molds can occur in warmer parts of the world and aflatoxicosis maybe produced in a wide range of tropical and subtropical food commodities such as figs, tree nuts and cereals. The most important crops are corn and groundnut, but it can also occur in temperate crops such as wheat. Although the production of aflatoxin initially was considered to be a problem in post harvest crops stored at inappropriate temperatures and water activities, it is now known that these compounds can be present in the field before harvest. *A.flavus* and *A. parasiticus* may infect healthy plants at a very early stage.

Biological effects: Aflatoxins are acute hepatotoxins and are known to be carcinogenic in some animal species as rat. Aflatoxin B_1 is acutely toxic to our species and is responsible for liver necrosis. The toxicological effect of the aflatoxins are influenced by their metabolism after intake into their body (Figure 10.1).

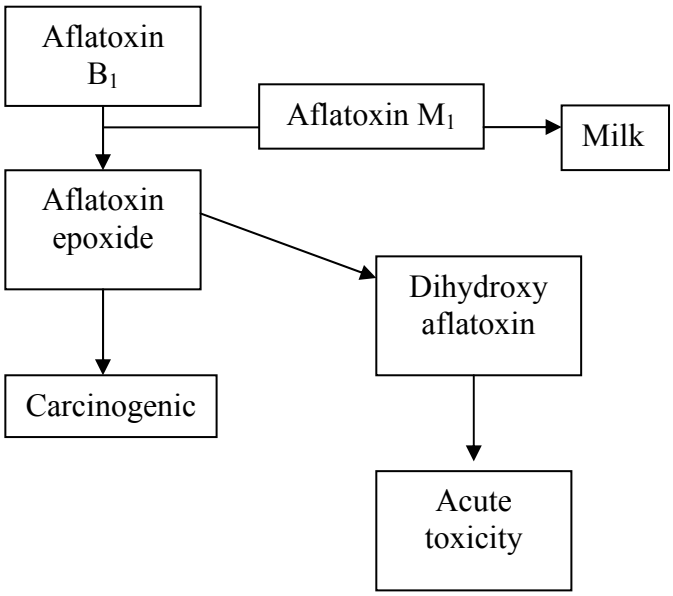

Figure 10.1: Representation of the metabolism of aflatoxin and its biological effects

When cows eat feed containing aflatoxin, aflatoxin M_1 and M_2 is excreted in the milk. Although M_1 and M_2 are less toxic than the parent compound B_1, M_1 retains its toxic and carcinogenic ability in many animals. The LD_{50} of AFB_1 for rats by the oral route is 1.2mg/kg and 1.5 to 2.0 mg/kg for AFG_1.

Control: Because aflatoxins are potentially widespread in occurrence and have an insidious combination of acute and chronic toxicity, it is prudent to control their presence in food. Many countries have legislation establishing maximum tolerance levels. Chemically treating the aflatoxin contaminated commodities like nuts maybe possible or to use technologically sophisticated equipment to sort and discard the contaminated units. It may also be possible to control the production of aflatoxin in the field by an integrated programme of agricultural management that may include plant breeding, improved irrigation and replacement of aflatoxigenic strains by non- aflatoxigenic strains of *A. flavus*.

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 mycotoxins? How are they harmful?

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2. Give the biological effects of aflatoxin.

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3. List the various natural toxins present in food.

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10.3.2 Ochratoxin

Occurrence: Ochratoxin A is a phenylalanyl derivative of a substituted isocoumarin produced by *Penicillium verrucosum* in temperate climate and by several species of Aspergillus in warmer and tropical parts of the world. *Penicillium verrucosum* is especially associated with stored cereals although it has also been isolated from meat and fish, however the occurrence of ochratoxin A in meat products is usually due to transmission into muscle, kidney and blood in animal fed on contaminated animal feed such as barley. Ochratoxin may also be transferred from dietary intake into milk. *Aspergillus ochraceus* is common on coffee beans, spices, soybeans, groundnut, rice and corn. Ochratoxin is heat resistant and is not destroyed by roasting or autoclaving though the ochratoxin producing fungi are capable of growth and mycotoxin production at temperature below 10ºC.

Biological effects: It is associated with the chronic progressive kidney disease in humans known as Balkan endemic nephropathy. There is increased evidence that it can also be considered as a carcinogen with genotoxic property as well as a potent nephrotoxin. The availability of improved methods of analysis has demonstrated that ochratoxin is quite widespread in foods and its presence in human body fluids confirms that there is a significant exposure with the human population. Ochratoxin is immunosuppressive and inhibits protein biosynthesis. Ochratoxin A has been classified by the International Agency for Reseach on Cancer (IARC) as a possible human carcinogen. Low doses as 70μg/kg body weight can induce kidney tumors in male rats. Its oral LD_{50} in rats is 20 to 22 mg/kg, and it is both hepatotoxic and neprotoxic.

Control: Once ochratoxin A has been formed in a food, it is difficult to remove by most forms of food processing. Cooking with or without previous soaking removes a significant amount of ochratoxin from beans but does not lead to total destruction. Beans still contain 16% to 60% of the initial ochratoxin contamination and it seems probable that the material may have leached out rather than destroyed.

10.3.3 Patulin

It is a toxic and antibiotic metabolite produced by several species of *Penicillin, Aspergillus* and *Paeciliomyces* but the most important in the context of human food production is *P. expansum*, a soft rot pathogen of apple and pears. Patulin is an unsaturated lactose and is sensitive to sulphur dioxide and is unstable in alkali but stable in acid.

Occurrence: It is found in a range of foods based on fruits. Presence of patulin in fruit juice is a indication that the juice was extracted from poor quality fruit which is undesirable and should be avoided with good manufacturing practices. This mycotoxin has also been found in moldy bread, sausage, fruits (including bananas, pears, pineapples, grapes and peaches) and other products.

Biological activity: Patulin has an acute oral LD_{50} in rodents of about 30-50 mg/kg and has been shown to be teratogenic, immunotoxic and neurotoxic and to cause gastrointestinal disturbances in rats. Patulin is quite rapidly excreted from animals. It causes chromosomal aberrations in animal and plant cells and is a carcinogen.

Control: In apples molded by *Penicillium expansum*, most of the patulin is confined to the region of damaged tissue and simply removing the lesions reduces the toxin by 90%, but if 1cm around the lesion is also removed, no patulin is detectable in rest of the apple. Ascorbic acid has been reported to reduce levels of patulin. Although pasteurization (using high temperature, short time treatment of ten seconds at 90ºC) causes some reduction in patulin in fruit juices, it is only of the order of 20%, which is not sufficient to make a badly contaminated food product acceptable.

10.3.4 Alternaria Toxins

Several species of Alternaria (*A.citri, A. alternata, A.solani* and *A. tenuissima*) produce toxic substances that have been found in apples, tomatoes, blueberries and others. The toxins produced include alternariol, alternariol monomethyl ether, altenuene, tenuazonic acid and altertoxin-I.

10.3.5 Citrinin

This mycotoxin is produced by *Penicillin citrinum*, *P.viridicatum* and other fungi. It has been recovered from polished rice, moldy bread, country cured hams, wheat, oats, rye and other similar products. It is a known carcinogen.

10.3.6 Penicillic Acid

This mycotoxin has biological properties similar to patulin. It is produced by a large number of fungi, including many Penicillia as well as members of the *A.ochraceus*. One of the best producers is *P.cyclopium*, it has been found in corn, beans and other field crops. Its LD_{50} in mice by subcutaneous route is 100 to 300 mg/kg and it is a proved carcinogen.

10.3.7 Sterigmatocystin

These mycotoxins are structurally and biologically related to the aflatoxins, and like the latter, they cause hepatocarcinogenic activity in animals. Among the organisms that produce them are *Aspergillus versicolor*, *A. nidulans*, *A.rugulosus*. The LD_{50} for rats by intra-peritoneal injection is 60 to 65 mg/kg.

10.3.8 Fusarium Toxins

Another important genus of mycotoxin producers is *Fusarium,* many species of which produce members of the trichothecene family of mold metabolites like deoxynivalenol, neosolaniol and T-2 toxin etc.

Deoxynivalenol

Deoxynivalenol (DON) is a far more common, but much less toxic, trichothecene and is produced by species such as *F. graminearum* and *F. culmorum*. LD_{50} of DON is 70mg/kg. The trichothecenes are remarkably stable compounds, and DON will survive both dry milling and wet milling processes of corn. The baking of bread has relatively little effect on trichothecenes such as DON.

Zearalenone

It was first isolated as the agent responsible for vulvovaginitis in pigs, has very little acute toxicity, but there should be some concern about chronic exposure to a compound known to be estrogenic. It may be produced, together with DON and other trichothecenes, in a wide range of cereals including corn, barley and wheat.

Moniliformin

It was first obtained from a strain of *Fusarium moniliforme* isolated from southern leaf blight- damaged corn seed as a water soluble toxin. The LD_{50} for mice has been reported to be 20.9 mg/kg for females and 29.1 mg/kg for males. At toxic doses moniliformin causes rapid death without obvious overt cellular damage, although acute degenerative lesions in the myocardium are reported.

Fumonisins

The most recently characterized mycotoxins of any major significance in human health are the fumonisins produced by species of *Fusarium*, such as *F. moniliforme*. Like a number of mycotoxins, the fumonisins are relatively heat stable and would not be significantly destroyed by drying processes for corn or heat treatments used for the production of maize derivatives. Fumonisin B_1 is water-soluble is known to be responsible for equine encephalomalacia, porcine pulmonary edema syndrome and hepatic cancer in rats and maybe involved in

the epidemiology of esophageal carcinoma in humans in southern Africa and parts of China.

The range of regulatory limits for mycotoxins

Mycotoxin	Regulatory Limit (µg/kg)
Aflatoxins in foods	0-50
Aflatoxin M1in milk	0-0.5
Deoxynivalenol in wheat	1000-4000
Ochratoxin A in foods	1-300
Patulin in apple juice	20-50
T-2 Toxin	100
Zearalenone	30-1000

10.4 BOTULISM

Botulism (Latin *botulus*, sausage) is a neuro-paralytic disease caused by the ingestion of food containing the neurotoxin produced by *Clostridium botulinum*.

10.4.1 Occurrence

Clostridium botulinum is an anaerobic, Gram-positive, spore forming, rod that produces the potent neurotoxin. The organism and its spores are widely distributed in nature and occur in both cultivated and forest soils, bottom of streams, lakes and coastal waters and in the intestinal tracts of fish and mammals and in viscera of shellfish.

On the basis of the serological specificity of their toxins, seven types of *Clostridium botulinum* are recognized: A, B, C, D, E, F and G. Types A, B, E, F and G cause disease in humans; type C causes botulism in fowls, cattle, mink and other animals and type D is associated with forage poisoning of cattle. Being a saprophyte, the organism seldom grows or produces toxic in the live animal; it can do so only by growing in food. The toxins are simple heat labile proteins and can be destroyed if heated at 80ºC for 10 minutes or longer.

C.botulinum does not produce the fully toxic molecule; instead a progenitor toxin is activated to its full toxicity by enzymes. The progenitor toxin is hydrolyzed to the highly toxic derivative toxin and is carried to target nerves where it binds to the synapses of motor neurons and prevents the release of the neurotransmitter acetylcholine. As a consequence, muscles do not contract in response to motor neuron activity and flaccid paralysis results.

10.4.2 Types and Symptoms

Different types of botulism are recognized: adult, infant and wound. A very small amount (a few nano grams) of toxin can cause illness.

Adult Botulism

Symptoms of botulism may develop anywhere between 12 and 72 hours after the ingestion of toxin containing foods. Symptoms include nausea, vomiting, fatigue, dizziness and headache, dryness of skin, mouth and throat, constipation, lack of fever, paralysis of muscles, double vision and finally respiratory failure

and death. The duration of the illness is from 1 to 10 or more days depending upon host resistance and other factors.

Infant Botulism

In the adult form of botulism, preformed toxins are ingested; in infant botulism, viable botulinal spores are ingested and upon germination in the intestinal tract, toxin is synthesized. It is confined to infants under a year of age. High number of spores are found in the feces of infants during the acute phase of the disease. It appears that ingested endospores, which maybe present in honey or other baby foods, germinate in the infants intestine. *C.botulinum* then multiplies and produces the exotoxin. The infant becomes constipated, listless, generally weak and eats poorly. Death may result from respiratory failure.

Wound Botulism

It is the rarest form of botulism. The illness results when *C.botulinum* by itself or with other microorganisms infects a wound and produces toxins which reach other parts of the body via the bloodstream. Foods are not involved in this type of botulism.

10.4.3 Diagnosis

Although botulism can be diagnosed by clinical symptoms alone, differentiation from other diseases maybe difficult. The most direct and effective way to confirm the clinical diagnosis of botulism in the laboratory is to demonstrate the presence of toxin in the serum or feces of the patient or in the food which the patient consumed.

10.4.4 Foods Implicated in Botulism

The types of foods involved in botulism vary according to food preservation and eating habits in different regions. Any food that is conducive to outgrowth and toxin production, that when processed allows spore survival, and is not subsequently heated before consumption can be associated with botulism. Almost any type of food that is not very acidic (pH above 4.6) can support growth and toxin production by *C.botulinum.* Botulinal toxin has been demonstrated in a considerable variety of foods, such as canned corn, peppers, green beans, soups, asparagus, mushrooms, spinach, tuna fish ham, sausage and smoked and salted fish.

10.4.5 Conditions Necessary for Outbreak

The following conditions are necessary for an outbreak of botulism:

1. Presence of spores of *C.botulinum* of type A, B or E in foods being consumed or being processed in some other way
2. A food in which the spores can germinate and the clostridia can grow and produce toxin
3. Survival of the spores of the organism eg: because of inadequate heating in canning or inadequate processing otherwise
4. Environmental condition after processing that will permit germination of the spores and growth and toxin production by the organism
5. Insufficient cooking of the food to inactivate the toxin
6. Ingestion of the toxin-bearing food

10.4.6 Prevention and Control

The prevention and cure of botulism involves:

1. Strict adherence to safe food-processing practices by the food industry
2. Educating the public on safe home-preserving (canning) methods for foods
3. Not feeding honey to infants younger than 1 year of age
4. Not tasting any processed food having a questionable odor
5. Recommended treatment for botulism includes early administration of botulinal antitoxin and intensive supportive care (including mechanical breathing assistance).

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 are the causative organism and the foods associated with botulinal food intoxication?

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2. Differentiate between adult and infant botulism.

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3. List down the various mycotoxins associated with food.

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10.5 STAPHYLOCOCCAL FOOD POISONING

Staphylococcal food poisoning results from consumption of food containing enterotoxin produced by enterotoxigenic strains of *Staphylococcus aureus*. It is caused by ingestion of improperly stored or cooked food (particularly foods such as ham, processed meats, chicken salad, pastries and ice cream) in which *S.aureus* has grown.

S.aureus is a Gram-positive coccus, very resistant to heat, drying and radiation. If the bacteria are allowed to incubate in certain foods, they produce heat-stable enterotoxin that render the food dangerous. Six different enterotoxins have been identified and are designated as A, B, C, D, E and F.

10.5.1 Occurrence

Staphylococci exist in air, dust, sewage, water, milk and food or on food equipment, environmental surfaces, humans and animals. Humans and animals are the primary reservoirs. Staphylococci are present in the nasal passages and throats and on the hair and skin of 50 percent or more of healthy individuals. A wide range of foods maybe involved in Staphylococcal food poisoning including ham, turkey, chicken and chicken salad, baked products, especially filled pastries, table ready-meats (sausage etc.), precooked frozen foods and dairy products.

S.aureus cells are relatively more resistant than many gram negative food spoilage organisms. Human intoxication is caused by ingesting enterotoxins produced in food by strains of *S.aureus,* usually because the food has not been kept hot enough (60ºC, or above) or cold enough (7.2ºC, or below). In frozen foods they may survive at –10ºC. In general, survival of *S.aureus* is best in foods that contain high concentration of sugars, eggs and buffering component such as phosphates and protein. Salt concentration less than 9.5%, temperature more than 20ºC and a pH in the range 6-8 are favourable for growth and enterotoxin formation.

10.5.2 Symptoms

A toxin dose of less than one micro gram in contaminated food will produce symptoms of staphylococcal intoxication. This toxin level is reached when *S. aureus* populations exceed 100,000 per gram. Symptoms of staphylococcal food poisoning usually develop with 1-6 hours of ingestion of contaminated food. Typical symptoms include severe abdominal pain, diarrhoea, vomiting, sweating, headache, prostration, nausea and sometimes a fall in body temperature. The mortality rate of staphylococcal food poisoning is negligible among healthy individuals.

10.5.3 Diagnosis

Diagnosis is based on the symptoms or laboratory diagnosis of the bacteria from leftover foods and from the stool cultures of victims. Enterotoxin maybe detected in foods by animal toxicity tests.

10.5.4 Foods Incriminated

Foods that are frequently incriminated in staphylococcal food poisoning include meat and meat products, poultry and egg products, egg, tuna, chicken, potato and macaroni, bakery products like cream-filled pastries, cream pies, chocolate eclairs, sandwich fillings, milk and dairy products. Foods that require considerable handling during preparation and that are frequently involved in staphylococcal food poisoning.

10.5.5 Conditions Necessary for Outbreak

The following conditions are necessary for an outbreak of staphylococcal food poisoning:

1. The food must contain enterotoxin producing staphylococci.
2. The food must be a good culture medium for growth and toxin production by the staphylococci.
3. The temperature must be favourable for growth of the cocci and enough time must be allowed for production of enterotoxin.
4. The enterotoxin bearing food must be ingested.

10.5.6 Prevention and Control

Staphylococcal food poisoning can be prevented by:

1. Avoiding contamination of food with *S.aureus.*
2. Prevention of growth of staphylococci by adequate refrigeration of foods and adjustment of more acid pH.
3. Killing staphylococci in susceptible foods by heating rapidly to 65-70ºC for 12-15 minutes.
4. Good personnel hygiene- exudates from skin lesions (pimples, boils) and nasal discharges of food handlers are rich sources of staphylococci and should be avoided.
5. Prolonged storage at room temperature of filled pastries, meat, salads and similar products that receive only a minimal heat treatment should be avoided.

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 are the conditions favouring the outbreak of Staphylococcal food poisoning?

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2. Give the preventive and control strategies of Staphylococcal food poisoning.

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10.6 LET US SUM UP

The unit deals with the food borne intoxications which are caused by ingestion of toxins produced from molds (*Aspergillus niger*, *Penicillium* sp.) or bacteria (*C.botulinum* and *S. aureus*). An effort has been made to point out the major conditions required for outbreak of the disease along with the diagnosis and the preventive measures required to prevent the outbreak of disease.

10.7 KEY WORDS

Food Borne Intoxication : It is the food borne disease caused due to ingestion of food containing the toxin.

Mycotoxins : These are secondary metabolites produced by filamentous fungi (molds) on food and feedstuffs that cause illness and death when ingested by man or animals.

Aflatoxin : This is the mycotoxin elaborated by *Aspergillus flavus*.

Ochratoxin : Mycotoxin produced by *Penicillium verrucosum*.

Patulin : Mycotoxin produced by species of *Penicillin, Aspergillus* and *Paeciliomyces*.

Citrinin : Mycotoxin produced by *Penicillin citrinum*.

Botulism : Food borne intoxication caused due to consumption of food containing toxin, produced by *Clostridium botulinum*.

Staphylococcal Food Poisoning : Food borne intoxication caused due to consumption of food containing toxin, produced by *Staphylococcus aureus.*

LD_{50} Dose : Lethal Dose of toxin at which minimum 50% of the population may get infected by the toxin.

Zearalenone : Mycotoxin produced by *Fusarium graminearum* and *F. tricinctum.*

10.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. • Mycotoxins are toxins (secondary metabolites) produced by filamentous fungi on food and feedstuffs that cause illness when ingested
   • Cause hepatotoxicosis, carcinogenesis, liver cirrhosis etc.
2. Biological effects of aflatoxin include hepatotoxicosis, carcinogenesis and liver cirrhosis.
3. Natural toxins in foods: Trypsin inhibitor, haemaglutenins, cyanogenic glycosides, saponins, alkaloids, goitrogens .

Check Your Progress Exercise 2

1. • Botulism is caused by the bacteria *Clostridium botulinum*.
   • All low acid foods can support growth and toxin production by *Clostridium botulinum*.
   • Unheated foods are causative agents e.g.: canned foods (canned corn, peppers, green beans, soups), sausages, smoked fish etc.
2. • Adult Botulism is prevalent amongst adults whereas Infant Botulism is prevalent in infants of less than one year of age.
   • Caused due to ingestion of viable spores of *C. botulinum.*
   • Symptoms in adults: Nausea, vomiting, fatigue, dizziness, headache, dryness of skin and respiratory failure
   • Symptoms in infants: Constipation, weakness and loss of appetite and in severe cases death
3. Mycotoxins associated with foods are:
   • Aflatoxins (produced by *Aspergillus flavus*)
   • Ochratoxin (produced by *Penicillium verrucosum*)
   • Patulin (*P. expansum*)
   • Citrinin (*Penicillin citrinum*)
   • Alternaria toxins (*Alternaria* sp.)
   • Penicillic acid (*P.cyclopium*)
   • Sterigmatocystin (*Aspergillus versicolor*)
   • Fusarium toxins (*Fusarium* sp)

Check Your Progress Exercise 3

1. Conditions necessary for outbreak of Staphylococcal food poisoning:

- Presence of viable staphylococcal bacteria in the food
- Growth and toxin production in food
- Toxin containing food must be ingested into the body

2. To prevent and control Staphylococcal food poisoning:
   - Avoid contamination of foods
   - Kill organism by heating, refrigeration
   - Personnel hygiene
   - Adequate cooking
   - Proper storage

10.9 SOME USEFUL BOOKS

1. Defigueiredo, M.P. and Splittstoesser, D.F. (1976) Food Microbiology: Public Health and Spoilage Aspects, AVI Publishing Co. Inc., Westport, Connecticut. pp 492.
2. Jay, J. (1996) Modern Food Microbiology, CBS Publishers, New Delhi. pp701.

UNIT 11 BACTERIAL FOOD INFECTIONS

Structure

11.0 OBJECTIVES

After reading this unit, you will be able to:

- describe major bacteria causes food born infections
- explain the mode of transmission of the food borne infection, symptoms and preventive measures.

11.1 INTRODUCTION

Food infection occurs when a pathogen enters the gastrointestinal tract and multiplies. Microorganisms can penetrate into the intestinal mucosa and grow there, or they can pass through other systemic organs. Infections are characterized by a delay in the appearance of gastrointestinal disturbance while the pathogen increases in numbers or affects invaded tissue. There is also usually a fever, one of the body's general responses to an infective organism.

Foodborne infections remain a major public health problem. The Council for Agricultural Science and Technology estimated in its 1994 report, *Foodborne Pathogens: Risks and Consequences*, that as many as 9,000 deaths and 6.5 to 33 million illnesses in the United States each year are food-related.

11.1.1 Zoonotic Diseases

The World Health Organization defines Zoonoses (Zoonosis, sing.) as "Those diseases and infections which are naturally transmitted between vertebrate animals and man".

Mode of transmission: Feces, urine, saliva, blood, milk, via aerosol, oral, contact with bedding or animals, etc.

Approximately 150 zoonotic diseases are known to exist. Wildlife serves as a reservoir for many diseases common to domestic animals and humans. Persons working with wildlife should be alert to the potential for disease transmission from animals. Generally, disease is more easily prevented than treated. Many zoonotic diseases are so common in nature, so rare in humans, or so mild in their symptoms, that wild animals pose a minimal health risk to people.

Zoonotic diseases include:

- Those which can be transmitted directly from animals to humans (e.g. rabies).
- Diseases that can be acquired indirectly by humans through ingestion, inhalation or contact with infected animal products, soil, water, or other environmental surfaces which have been contaminated with animal waste or a dead animal (e.g. salmonellosis, leptospirosis, anthrax). *Campylobacter* infection is mainly found in chicken meat. Listeriosis and *E.coli* gastroenteritis are two other common infections caused by zoonotic agents. All these will be discussed one by one.
- A disease which has an animal reservoir, but requires a mosquito or other arthropod to transmit the disease to humans (e.g. St. Louis encephalitis, Rocky Mountain spotted fever).

11.2 SALMONELLOSIS

Salmonellosis is the most reported zoonotic disease in European countries. Salmonellosis (*Salmonella* gastroenteritis) results from the ingestion of foods that contain significant numbers of viable cells of the members of the genus *Salmonella*. It is the most frequently occurring food borne infection.

Salmonella are small gram negative, motile, non-spore forming rods that ferment glucose, usually with gas, but usually do not ferment lactose or sucrose. They are widely distributed in nature, with humans and animals being their primary reservoir. Generally large number of salmonellae typically 10^6 to 10^9 bacterium must be ingested to cause illness.

Occurrence: The initial source of the bacteria is the intestinal tract of animals such as birds, reptiles, farm animals, humans and occasionally insects. As intestinal form, the organisms are excreted in feces from which they maybe transmitted by insects and other living creatures to a large number of places, polluted water and contaminated food. The organism may get transferred from actual infected cases of the disease or from carriers. A carrier is defined as a person or an animal that repeatedly sheds bacteria, usually through feces, without showing any signs or symptoms of the disease. Infected rodents, rats and mice may contaminate unprotected foods with their feces and thus spread *Salmonella* bacteria. Flies may play an important role in the spread of *Salmonella*, especially from contaminated fecal matter to foods. Humans acquire the bacteria from contaminated food such as beef products, poultry, eggs, egg products or water.

Symptoms: The susceptibility of humans varies with the species and strains of the organism and the total number of bacteria ingested. A longer incubation period usually distinguishes salmonellosis from staphylococcus poisoning: usually 12-36 hours for the former and about 2-4 hours for the latter. The

principle symptoms of a salmonella gastroenteritis infection are nausea, vomiting, abdominal pain and diarrhoea that usually appear suddenly. This may be preceded by a headache and chills. Other evidences of the disease are watery, greenish-fowl-smelling stools, prostration, muscular weakness, faintness, usually a moderate fever, restlessness, twitching and drowsiness. The mortality is less than 1%. Intesibility may vary from slight discomfort and diarrhoea to death in 2 to 6 days. About 0.2 to 5.0% of the patients may become carriers of the *Salmonella* organism. During the acute phase of the disease, as many as one billion salmonellae can be found per gram of feces.

Associated foods: Raw meats, poultry, eggs, milk and dairy products, fish, shrimp, coconut, sauces and salad dressings, cake mixes, cocoa, peanut butter and chocolate.

Conditions Necessary for Outbreak

The food must contain or become contaminated with the *Salmonella* bacteria.

These bacteria must be there in considerable numbers i.e., food should be a good culture media, temperature favourable and enough time allowed for appreciable growth.

The viable organism must be ingested.

Prevention of Outbreak: The control of food borne salmonella infection requires the following:

1. Preventing food contamination by human carriers, especially food handlers.
2. Avoiding the use of animal products from domestic livestock that are grossly infected with salmonellae.
3. Avoiding the use of food ingredients that contain salmonellae.
4. Processing all foods susceptible to *Salmonella* contamination at time-temperature schedules sufficient to destroy the organism. Heating foods so that all portions reach 66ºC for 12-15 minutes will assure destruction of even most resistant *Salmonella* types.
5. Refrigerating all foods susceptible to *Salmonella* contamination and avoiding prolonged holding of these foods at room temperature.

11.3 ENTEROPATHOGENIC *ESCHERICHIA COLI*

Escherichia coli is generally regarded as part of the normal flora of the human intestinal tract and that of many animals. Serotypes of *E. coli* which have been implicated in human diarrhoeal diseases or food poisoning outbreaks and have been designated enteropathogenic *E. coli* (EEC). They grow over a wide range of temperatures, 20-40ºC with a minimum growth temperature at 10ºC and an optimum at 37ºC. Heating at 65ºC for 15-20 minutes is lethal. The pH range for growth is 4.2-8.50, with an optimum in the range of pH 7.2-7.5. *E. coli* will grow in the presence of 5.0% salt at 37ºC but 10% is inhibitory.

Symptoms: The *E. coli* gastroenteritis syndrome is caused by the ingestion of 10^6-10^{10} viable cells/g that must colonize the small intestine and produce enterotoxin. The syndrome is characterized primarily by non-bloody, watery diarrhoea without inflammatory exudates in stools. Incubation time of disease

is around 2 days after eating the contaminated food and may last for 8 days. Common symptoms included are cramps, chills, vomiting, aches and headache.

Associated Foods: *E. coli* is the etiologic agent of food poisoning involves variety of foods such as cream pie, mashed potatoes, cream puffs and creamed fish. Other *E. coli* food poisoning outbreaks have been attributed to the consumption of milk, cheese, ice cream, meats, fish and macaroni. *E. coli* is relatively sensitive to destruction by drying or freezing but some survivors may exist for extended periods.

"Enteropathogenic" strains colonize in the small intestine and cause acute gastroenteritis in newborns and in infants up to two years of age. "Enteroinvasive" strains invade the epithelial cells of large intestine and cause diarrhoea in older children and adults. "Enterotoxigenic" (enterotoxin producing) strains produce one or both of two different toxins:a heat stable toxin (ST) and a heat labile toxin (LT). Both toxins cause diarrhoea in adults and infants. Enterotoxigenic strains of *E. coli* are often associated with Travellers' diarrhoea, a common disease contracted by tourists when visiting developing countries. Diagnosis of travellers' disease is based on the past travel history and symptoms. Laboratory diagnosis is by isolation of the bacteria from feces. Treatment is with fluid and electrolytes. Other strains of *E. coli* which are usually harmless in their normal habitat (the intestine) can cause disease when they gain access to other sites or tissues. These diseases include urinary tract infections, septic infections, bacteremia, meningitis, pulmonary infections, abscesses, skin and wound infections.

Prevention and Control: Involves avoiding contaminated food and water that have high coliform counts, avoiding unpasteurized juices, washing fresh fruits and vegetables thoroughly before eating raw, using adequate cooking procedures for destruction and prompt refrigeration. Most people recover from *E. coli* infection within 5-10 days without treatment. Antibiotics and antidiarrhoeal drugs are usually not helpful.

11.4 *BACILLUS CEREUS* GASTROENTERITIS

Bacillus cereus is not a common cause of food poisoning. It is a Gram positive, aerobic, spore forming rod shaped bacteria normally present in soil, dust and water. The bacterium has a minimum growth temperature around 4-5ºC, with maximum around 48-50ºC. Optimum pH range for growth is 4.9 to 9.3.

Symptoms: Extremely large numbers (10^8 per gram) of viable cells of *B.cereus* must be ingested to develop signs and symptoms of the syndrome. The bacterial cells produce intoxication characterized by acute abdominal pain, flatulence and watery diarrhoea. Headache and dizziness are common, dehydration and prostration may occur but nausea, vomiting, fever and chills are rare. The illness appears within 6-15 hours after consumption of food and the symptoms usually last less than 24 hours.

Associated Foods: Vehicle foods consist of cereal dishes that contain corn and corn starch, mashed potatoes, vegetables, minced meat, liver sausage, milk, cooked meat. Food mixtures such as sauces, puddings, soups, pastries and salads have frequently been incriminated in outbreaks.

11.5 CHOLERA

Cholera is caused by the gram negative, *V. cholerae*, which is acquired by ingesting food or water contaminated by fecal material from patients or carriers (shellfish and plankton may be the natural reservoir).

Symptoms: Once the bacteria enter the body, the incubation period is from several hours to three or more days. An infective dose of around one million organisms should be ingested to cause illness. The bacteria adhere to the small intestine wall, where they secrete the cholera enterotoxin, choleragen. As a result, there is hyper secretion of water and chloride ions, while inhibiting absorption of sodium ions. The patient experiences an outpouring of fluid and electrolytes with associated abdominal muscle cramps, vomiting, fever and watery diarrhoea. The diarrhoea can be so profuse that a person can lose 10-15 liter of fluid during the infection. Death may result from the elevated concentration of blood proteins, caused by reduced fluid levels, which leads to circulatory shock and collapse. Onset of the illness is generally sudden, with incubation periods varying from 6 hours to 5 days.

Associated Foods: Cholera is generally a disease spread by poor sanitation, resulting in contaminated water supplies. Sporadic cases occur when shellfish harvested from fecally polluted coastal waters are consumed raw.

Diagnosis: Cholera can be confirmed only by the isolation of the causative organism from the diarrheic stools of infected individuals.

Prevention: Following recommendations are there to prevent cholera outbreak:

- Drink only water that you have boiled or treated with chlorine or iodine. Other safe beverages include tea and coffee made with boiled water and carbonated, bottled beverages with no ice.
- Eat only those foods that have been thoroughly cooked and are still hot, or fruit that you have peeled yourself.
- Avoid undercooked or raw fish or shellfish.
- Make sure all vegetables are cooked, avoid salads.
- Avoid foods and beverages from street vendors.

A simple thumb rule is **“Boil it, cook it, peel it, or forget it”**.

Control: Individuals infected with cholera require oral rehydration therapy with NaCl plus sucrose, sodium bicarbonate and potassium chloride to stimulate water uptake by the intestine. The antibiotics of choice are a tetracycline or aproflaxin. The most reliable control methods are based on proper sanitation, especially of water supplies. The mortality rate without treatment is often over 50%. Medical treatment to prevent dehydration prevents all complications.

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. Briefly discuss the Salmonella food infection.

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2. How do you prevent food borne infection?

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11.6 *VIBRIO PARAHAEMOLYTICUS* GASTROENTERITIS

While most other known food poisoning syndromes may be contracted from a variety of foods, *V. parahaemolyticus* gastroenteritis is contracted almost solely from seafood. It can grow in presence of 1-8% NaCl, in pH range 9.8-11.0 with 7.6-8.6 being optimum.

Symptoms: A total of greater than one million organisms may cause disease. Symptoms of intoxication which range from mild to severe and fatal, include abdominal pain, which maybe intense; a burning sensation of the stomach; vomiting and diarrhoea with watery stools and sometimes bloody discharges; fever. The mean incubation period is in range of 3-76 hours after the ingestion of the organism.

Associated foods: Vehicle foods for outbreak are raw, improperly cooked, or cooked, recontaminated seafoods, such as, oysters, shrimps, crabs, lobsters, clams and related shellfish. Cross-contamination may lead to other foods as vehicles. Improper refrigeration of seafoods contaminated with this organism will allow its proliferation, which increases the possibility of infection.

Diagnosis: Diagnosis of gastroenteritis caused by this organism is made by culturing the organism from the diarrheic stools of an individual.

Prevention: Consumption of raw or improperly cooked seafoods should be avoided as they are susceptible to infection by *V. parahaemolyticus.*

11.7 SHIGELLOSIS

Shigellosis or bacterial dysentery, is caused by facultatively anaerobic, gram-negative, non-spore forming, rod-shaped organisms belonging to the genus *Shigella* within the family enterobacteriacae. In general, shigellosis is a self-limiting disease, lasting 5 to 6 days if untreated, however in young malnourished children, the elderly and the immuno compromised (eg, AIDS patients), the disease may be fatal. It is estimated that shigellosis is responsible for the death of 500,000 children worldwide each year. There are many points of similarity between *Shigella* and *Salmonella*. They dwell primarily in the gastrointestinal tract, with optimum temperature of 37ºC, grow both aerobically and anaerobically they grow freely in warm, bland, moist foods. But unlike salmonellae, the shigellae have no flagella and thus are non-motile. The species involved are *Shigella sonnei, S. dysenteriae, S. flexineri* and *S. boydii*. As few as 10cfu of *S. dysenteriae* are known to initiate infection in susceptible individuals. The illness caused by *Shigella* accounts for less than 10% of the reported outbreaks of food borne illness in US. The organisms tolerate salt concentration of 5-6% and are relatively heat sensitive.

Occurrence: Poor personal hygiene is a common factor in food borne shigellosis, with shellfish, fruits and vegetables, chicken and salads being prominent among vehicle foods. The prominence of these foods is due to the fecal-oral route of transmission. Outbreaks have been also traced to foods such as chocolate pudding, salads.

Symptoms: Pathogenicity involves the release of lipopolysaccharide endotoxin which infects the intestinal mucosa. Shigellosis ranges from fairly mild to very severe and fatal. The onset is usually abrupt, requiring from 1-7 days of incubation, but sometimes requiring as many as 14 days. Symptoms are abdominal pain and cramps caused by inflammation of mucus surface of large intestine, nausea, diarrhoea, vomiting, elevated temperature. The mortality associated with *S. dysenteriae* infection is around 20% but it is much lower with other species. In severe instances, excessive diarrhoea leads to electrolytic imbalance in the bloodstream and ulceration in large intestine. There may be kidney failure, jaundice and persistent internal bleeding. The infection is localized and organs other than the large intestine are not invaded.

Diagnosis: Serological identification of culture isolated from stool helps to diagnose the disease.

Prevention and Control: The control of *Shigella* food borne infection is similar to that of salmonellae; avoiding contamination of foods by animal or human carriers or their excrement, thorough cooking and prompt cooling. Proper personal hygiene should be maintained. In severe cases of shigellosis, dehydration of the body may necessitate intravenous replacement of fluid with electrolytes. Ampicillin antibiotic can decrease the duration of the disease.

11.8 CAMPYLOBACTERIOSIS

It is caused by *Campylobacter jejuni*, a Gram negative rod. It is a microaerophillic organism, which means it has a requirement for reduced levels of oxygen. It is often isolated from healthy cattle, chickens, birds and even

flies. It is also sometimes present in non-chlorinated water sources such as streams and ponds. The bacteria cause between 5 and 14 per cent of all diarrhoeal illnesses worldwide. C. jejuni primarily affects children under 5 years old and young adults (15-29 years old).

Symptoms: *C. jejuni* infection causes diarrhoea, which may be watery or sticky and can contain blood and fecal leukocytes (white cells). Other symptoms often present are fever, abdominal pain, nausea, headache and muscle pain. The illness usually occurs 2-5 days after ingestion of the contaminated food or water. Illness generally lasts 7-10 days, but relapses are not uncommon (about 25% of cases). Most infections are self-limiting and are not treated with antibiotics. The infective dose of *C. jejuni* is considered to be 400-500 bacteria.

Associated Foods: *C. jejuni* frequently contaminates raw chicken. Survey shows that 20-100% of retail chickens are contaminated. Many healthy chickens carry these bacteria in their intestinal tracts. Raw milk is also source of infections. The bacteria are often carried by healthy cattle and by flies on farms. Non-chlorinated water may also be a source of infection.

Prevention: the various ways to prevent campylobacteriosis are:

- Wash hands before preparing foods.
- Wash hands immediately after handling raw poultry or other meat.
- Proper cooking of chicken to internal temperature of 170ºC.
- Drink pasteurized milk and chlorinated water.
- Wash hands after handling pet feces or visiting zoos.

Control: If a person is suffering from campylobacteriosis, he can take an antibiotic such as ciproflaxin or azithromycin. Erythromycin also helps to treat the diarrhoea. Those having diarrhoea should take plenty of water.

Check Your Progress Exercise 2

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

1. Give the causative organism and symptoms of Bacillary Dysentry.

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2. What are the symptoms and foods associated with campylobacteriosis?

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11.9 YERSINIOSIS (*YERSINIA ENTEROLYTICA* INFECTION)

In the genus Yersinia, 11 species are recognized, including *Y. pestis*, the cause of plague. The species of primary interest in foods is *Y. enterolytica*.

Occurrence: *Y. enterolytica* is widely distributed in the terrestrial environment and in lake, well and stream waters which are sources of warm-blooded animals. It is more animal adapted and is found more often among human isolates than the other species.

Animals from which *Y. enterolytica* has been isolated include cats, birds, dogs, beavers, guinea pigs, rats, camels, horses, chickens, deer, cattle, swine, fish and oysters. It is widely believed that swine constitutes the single most common source of *Y. enterolytica* in humans.

In addition to gastroenteritis, this organism has been associated with human pseudoappendicitis, mesenteric lymphadenitis, reactive arthritis, colon and neck abscesses and cholecystis. It has been recovered from urine, blood, cerebrospinal fluid and the eyes of infected individuals. It is also recovered from the stools of gastroenteritis victims.

Associated Foods: The organism has been isolated from cakes, vacuum-packaged meats, seafood, vegetables, milk and other food products. It has been isolated also from beef, lamb and pork. Of all sources, swine appears to be the major source of pathogenic for humans.

Symptoms: Symptoms of the gastroenteritis syndrome develop several days following ingestion of contaminated foods and are characterized by abdominal pain and diarrhoea. Children appear to be more susceptible than adults and the organism may be present in stools for up to 40 days following illness. A variety of systemic involvements may occur as a consequence of the gastroenteritis syndrome.

The usual symptoms, including severe abdominal pain, fever and diarrhoea occur 24 to 36 hours after consumption of the product. The abdominal discomfort is quite specific and usually manifests itself as a sharp pain in the lower right quadrant of the abdomen. For this reason it has frequently been described as pseudoappendicitis.

Although the organism has been isolated from many foods, there have been relatively few food-borne outbreaks attributed to *Y. enterolytica*. The isolation from pasteurized milk is probably the result of post pasteurization contamination. The unique characteristic of the organism is its ability to grow at commercial refrigeration temperatures, i.e. less than 5ºC.

11.10 *LISTERIA MONOCYTOGENES* INFECTION (LISTERIOSIS)

Listeria monocytogenes is a gram-positive, motile, non-sporing rod. It is widely distributed in nature and can be found on decaying vegetation and in soils, animal feces, sewage, silage and water. In cattle it can result in abortion and mastitis and the infected animals shed the organism in milk. Other infected organisms including sheep and chicken can serve as source of the organism in the food supply.

Syndrome: Listeriosis in humans is not characterized by a unique set of symptoms since the course of the disease depends upon the state of the host. Non-pregnant healthy individuals who are not immunosuppressed are highly resistant to infection by *L. monocytogenes*. When susceptible adults contract the disease, meningitis and sepsis are the most commonly recognized symptoms. Pregnant females who contract the disease may not present any symptoms, but when they do, they are typically mild and influenza-like. Abortion, premature birth or still birth is often the consequence of listeriosis in pregnant females. When a newborn is infected at the time of delivery, listeriosis symptoms typically are those of meningitis and they begin at 1 to 4 weeks after birth, although a four week incubation has been recorded. The usual incubation time in adults ranges from one to several weeks.

Since *L. monocytogenes* can grow over the temperature range of about 1º to 45ºC and the pH range 4.1 to around 9.6, it maybe expected to survive in foods for long periods of time.

Some of the ways in which *L. monocytogenes* is disseminated throughout the environment, along with the many sources of the organism to humans, are illustrated below.

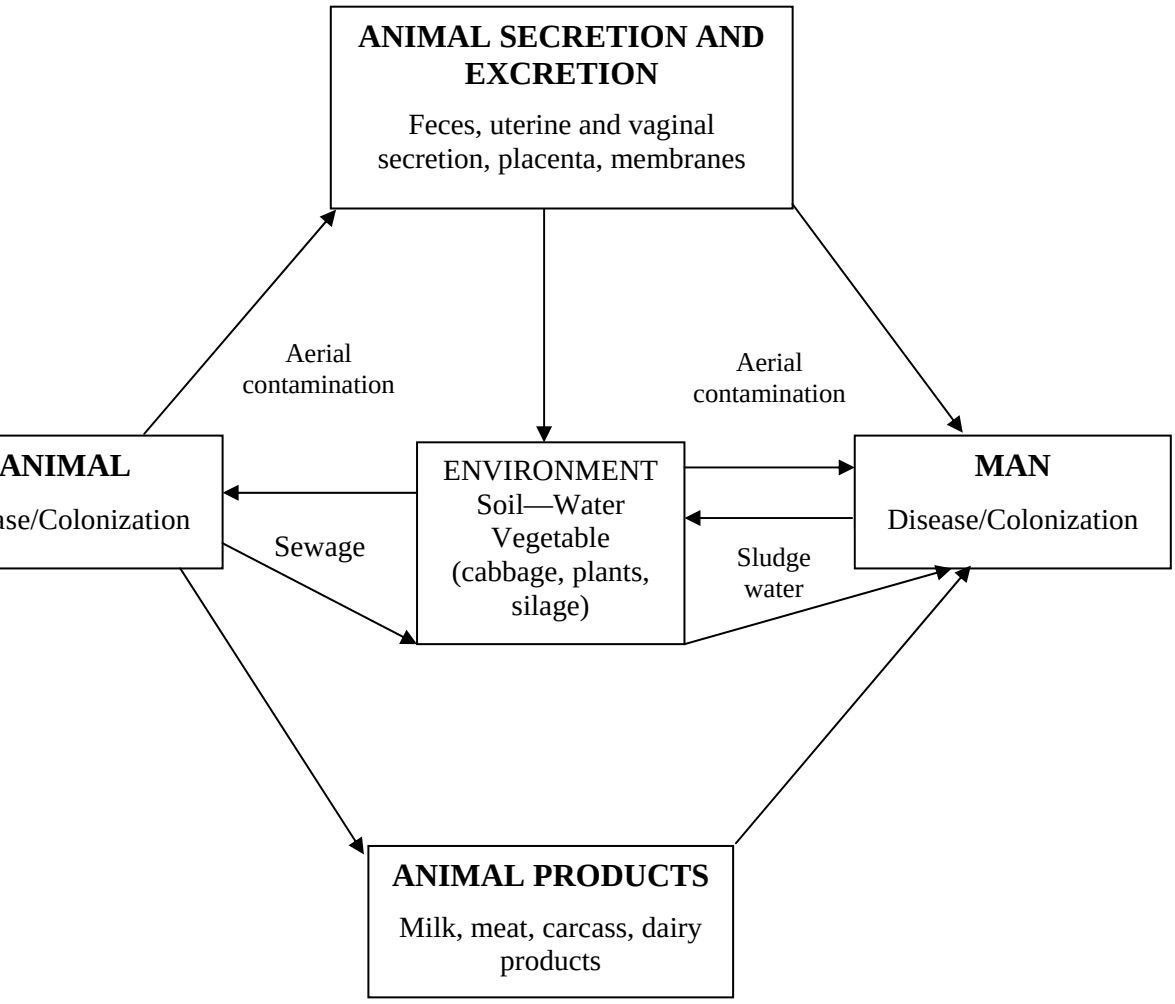

Figure 13.1: Ways in which *L. monocytogenes* is disseminated in the environment, animals, foods and humans

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 yersiniosis? Give its symptoms.

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2. How is *L. monoctogenes* infection transmitted?

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Tips on Foodborne Illness Prevention for Consumers

Salmonella, Escherichia coli, Shigella, Campylobacter, Listeria, and the list goes on. Interesting names for little organisms that can cause big bad health problems. Bacterial foodborne diseases have become an acute public health crisis in this country, responsible for about 6.5 to 81 million illnesses and 9,000 deaths per year. While it is unrealistic to think there would ever be a zero level of contamination in our food supply, 90% - 95% of all foodborne bacterial illness is preventable. You cannot see, taste, or smell most bacterial foodborne problems, so here's what you can do to safeguard your family.

In General:

- Don't ever consume products such as unpasteurized milk or unpasteurized apple cider or other foods made with unpasteurized products. Thoroughly cook all meat, poultry and fish products. Meats are thoroughly cooked at 71ºC, poultry at 82ºC, and fish when they easily flake with a fork.
- Break open any hamburger patties (other ground meat dishes such as meatballs, too) prior to ingestion to make sure there is no pink meat and that the juices run clear.

When Dining Out

- Hot food should be served hot and cold food should be served cold or send it back.
- Talk to the restaurant manager. Find out how much importance they place on sanitary and bacterial issues. Do they routinely use thermometers? Do

they own and routinely use a thermocouple which is a special thermometer used to accurately test temperatures of thin food? If you receive undercooked food (especially ground meat) express your displeasure and nicely inform them of the risks. Let them know how important food safety is to you.

When Shopping

- Take great care to avoid dripping raw juice from meat, poultry or fish onto your hands or other foods, especially produce. Raw juices often contain bacteria.
- Shop for cold and frozen foods last and take them immediately home to the refrigerator or freezer. Use ice chests in your car during transport, especially in the summer months or when running errands.
- Buy food only if it is in good condition. Frozen foods should be solid, refrigerated case food should be well-chilled, and canned goods should be free of dents and bulging lids. Point out any problems to the store manager.

When in your Kitchen

- Always wash your hands in hot soapy water before food preparation and after handling raw meat, poultry or fish.
- Keep your refrigerator's temperature as cold as possible without freezing your milk or produce (approximately 4°C). Keep your freezer's temperature cold enough to keep frozen food rock hard (approximately -18°C). If you are ever in doubt, temperatures can be checked with an appliance thermometer.
- After shopping, put any fresh meat, poultry, or fish, which won't be use within the next few days directly into the freezer.
- Thaw frozen food in the refrigerator or in a microwave followed by immediate cooking. ***Do not thaw food at room temperature on the counter***.
- Take great care to avoid dripping of raw meat, poultry, or fish juices onto or into other foods in the refrigerator. Use plates, platters or containers under them if necessary.
- Never put cooked food back on a plate/container which has had fresh juices on it. For example, when barbecuing take out an extra platter to put the grilled food on.
- Use non-porous plastic cutting boards for preparation and cutting of meat, poultry, and fish.
- Wash all cutting boards surfaces, platters, and containers which fresh meat, poultry, and fish have come in contact with, in hot, soapy water thoroughly before using for other foods.
- Avoid cross contamination by washing kitchen towels after contact with raw juices and by replacing sponges often. *Use paper towels wherever you can.* A good disinfectant for utensils and countertops is one tablespoon household bleach in few litres of water.

- When using eggs, cook them until firm. Don't use recipes calling for only partially cooked eggs. For example, raw cookie dough could be dangerous.

When using the microwave remember there can be cold spots, so stir and rotate food for thorough cooking.

11.11 THE MOST IMPORTANT POINT TO REMEMBER IS TO WASH YOUR HAND

Preferably with antibacterial soap for at least 20 seconds....

- ***before food preparation***
- ***after fresh meat, poultry, or fish handling and before you then touch other food, eat, or prepare baby bottles***
- ***after using the bathroom***
- ***after changing diapers***
- ***after helping toddlers in the bathroom***
- ***after cleaning the bathroom***
- ***after handling pets, cleaning litter boxes or dog runs***

PLEASE REMEMBER: Foodborne bacterial illness can be very contagious. This is called secondary transmission where a person gets ill not from ingesting the contaminated food but from coming in close contact with someone who has. Secondary transmission has been documented in the home, in day care centres, in preschools, in schools, in hospitals, and in senior citizen facilities. When someone you know has diarrhoeal illness, use extreme sanitary measures, to guard against the spread of the disease. The use of anti-diarrhoeal medication for treatment of foodborne bacterial diarrhoea is not recommended and in some cases can be harmful. If symptoms are severe, see your doctor. If food poisoning is suspected, call your local health department. Your prompt action could help prevent someone else from getting ill.

11.12 LET US SUM UP

In this unit, we attempted to familiarize you with certain outbreaks of bacteria which are responsible for food borne infections. We hope that you will be able to know the various bacteria which cause food borne infections. You will also be able to differentiate in the symptoms of the diseases caused and list the main reasons for the outbreak of the infections. This unit would also have helped you to know the information regarding the prevention of the outbreaks of the disease.

11.13 KEY WORDS

Food Infection : Food borne disease caused due to ingestion of large number of viable organisms which cause disease.

Zoonotic Diseases : Those diseases and infections which are naturally transmitted between vertebrate animals and man.

Salmonellosis	**:**	Food borne infection caused by *Salmonella gastroentritis*.
Enterotoxigenic *E. coli*	**:**	*E. coli* which produce toxins in the intestine.
Cholera	**:**	Food borne infection caused by *Vibrio cholarae*.
Shigellosis	**:**	Food borne infection caused by *Shigella sonnei, S. dysenteraei*.
Yersiniosis	**:**	Food borne infection caused by *Yersinia enterolytica*.
Listeriosis	**:**	Food borne infection caused by *Listeria monocytogenes*.

11.14 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1.
   - Salmonella food infection is caused by *Salmonella gastroenteritis*.
   - Transmitted by fecal contamination of foods.
   - Incubation period 12-36 hours.
   - Symptoms: nausea, vomiting, diarrhoea, abdominal pain and green watery stools.
   - About 10^6-10^9 organisms must be ingested to cause infection.
   - *Salmonellosis* prevented by: prevention of contamination of food by Salmonella, avoiding intake of contaminated food and by destruction of organism at 66ºC for 12-15 min.
2. Prevention of Food borne infections:
   - Avoid consumption of contaminated foods and water
   - Eat properly cooked foods
   - Wash raw fruits and vegetables properly
   - Proper hygiene

Check Your Progress Exercise 2

1.
   - Bacillary dysentery is caused by *Shigella* sp. (*Shigella sonnei, S. dysenteraei).*
   - Incubation period is about 1 to 7 days.
   - Causes abdominal pain, cramps, inflammation of intestinal mucousa, diarrhoea, vomiting, nausea and fever.
   - In severe cases; intestinal bleeding, electrolytic imbalance, ulceration, kidney failure and jaundice.

2. • Campylobacteriosis incubation period: 2-5 days after ingestion of contaminated food.

- Symptoms: Diarrhoea containing blood, fever, abdominal pain, nausea, headache and muscle pain.
- Transmission of infection by raw chicken, raw milk and non-chlorinated water.

Check Your Progress Exercise 3

1.
   - Yersiniosis caused by the bacteria *Yersinia enterolytica*.
   - Causes severe abdominal pain, fever and diarrhoea.
2.
   - Listriosis caused by *Listeria monocytogenes* infection.
   - Transmitted by animal excretions (fecal matter) and secretions, infected vegetables, aerial contamination, sewage, sludge, polluted water, rivers and infected animal products like milk and milk products, meat, fish etc.

11.15 SOME USEFUL BOOKS

1. Ayers, J.C., Mundt, J.O., Sandine, W.E. (1980) Microbiology of Foods, W.H Freeman and Co., San Franscisco. pp708.
2. Frazier, W.C. and Westoff, D.C. (1988) Food Microbiology, Tata McGraw-Hill Publishing Co., New Delhi. pp 539.
3. Pelczar, M.J., Chan, E.C.S., Kreig, N.R. (1997) Microbiology, Tata McGraw-Hill Publishing Co Ltd, New Delhi. pp 918.

UNIT 9 FOOD BORN DISEASES

Structure

9.0 OBJECTIVES

After reading this unit, you will be able to:

- know the food borne diseases;
- learn to differentiate between food borne intoxications and food borne infections;
- explain that the food borne diseases may be caused due to microorganisms, both bacterial and non bacterial or chemicals;
- explain why investigation of food borne disease outbreak is necessary; and
- describe how the investigation of food borne disease outbreak is carried out.

9.1 INTRODUCTION

Food borne disease (FBD) is caused by consuming contaminated foods or beverages. In addition poisonous chemicals or other harmful substitutes can cause food borne diseases if they are present in food. More than 250 different food borne diseases have been described. A classification of food borne diseases is given in Figure 9.1. Food borne diseases may be intestinal diseases but can be other type as well.

9.2 TYPES OF FOOD BORN DISEASES

With regard to their epidemiology, they can be divided into two major categories (Figure 9.2):

i) **Food borne Intoxication:** Examples include botulism or staphylococcal food poisoning, the causative microorganism produces an exotoxin in food: when a person consumes the food, the toxin is ingested and gives rise to disease.

ii) **Food borne Infections:** the causative organisms are ingested: these subsequently grow within the body and cause damage.

Both infections and intoxications often cause diarrhoea. Severe diarrhoea accompanied by blood or mucus is called dysentery. Both types of digestive system diseases are also frequently accompanied by abdominal cramps, nausea and vomiting. Diarrhoea and vomiting are both defensive mechanisms designed to rid the body of harmful material.

The general term gastroenteritis is applied to disease causing inflammation of the stomach and intestinal mucosa.

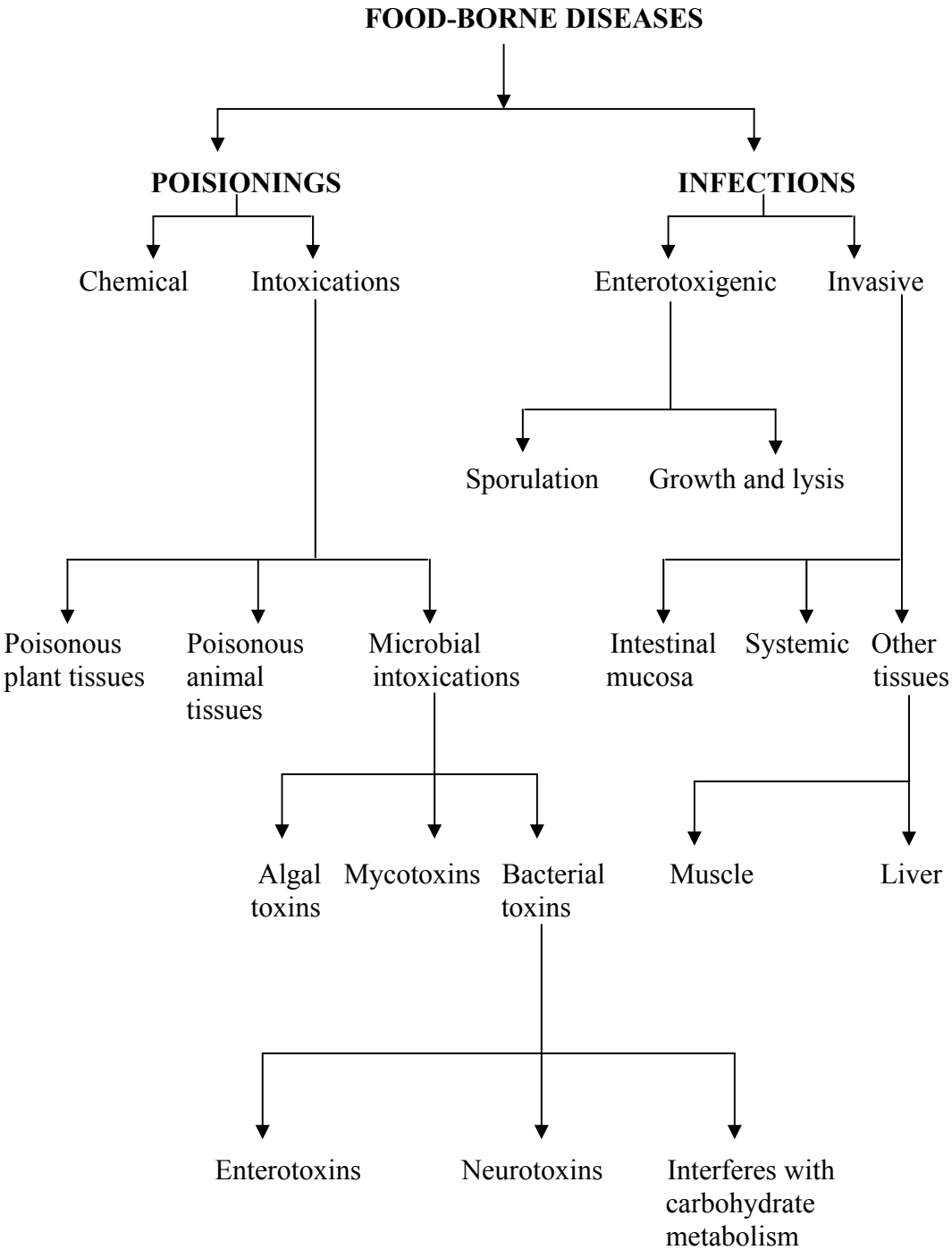

Figure 9.1: Classification of food borne diseases

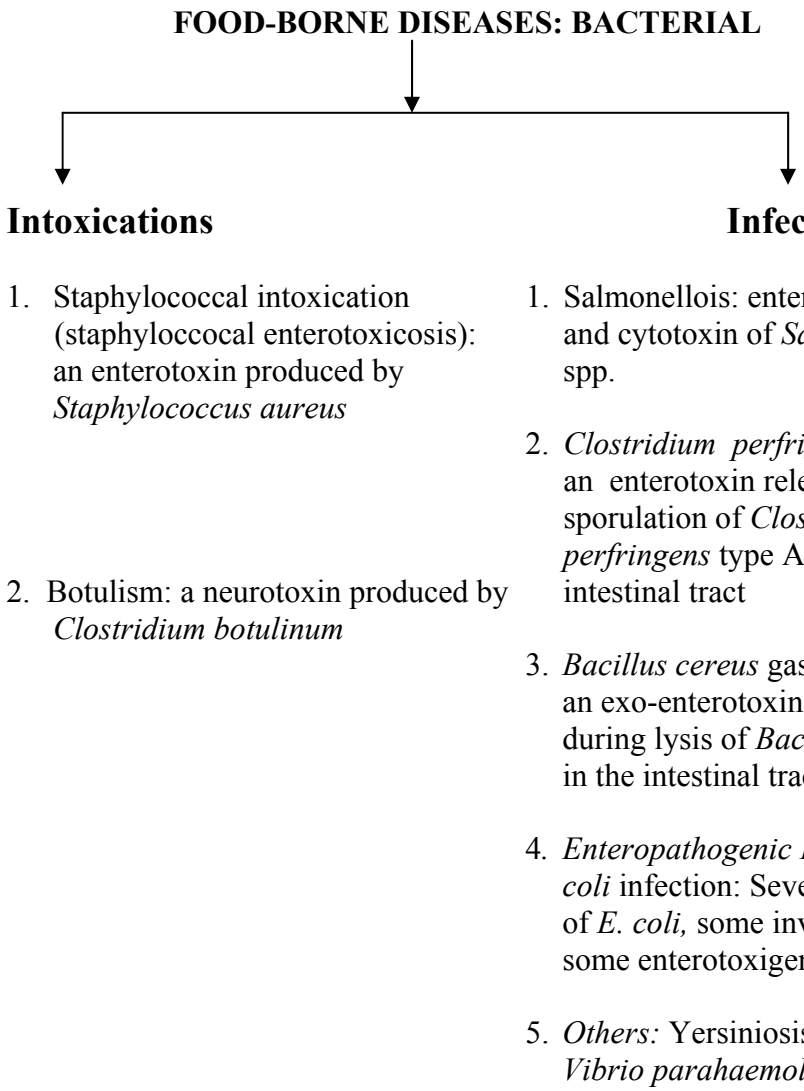

Figure 9.2: Bacteria responsible for food borne intoxications and infections

9.3 HUMAN DISEASES

Microorganisms that produce infectious diseases in human are categorized as pathogens, because they have the ability to injure body tissues and/or alter body functions.

In general, pathogenic microorganisms express their disease-producing properties through two kinds of mechanisms: (1) invasion of tissues (invasive microorganisms) and (2) production of toxins (toxigenic microorganisms).

Invasive pathogens have the ability to produce and excrete one or more kinds of extracellular enzymes resulting in injury to host tissues. Toxigenic microorganisms produce toxins of two types:

- Exotoxins – produced within certain kinds of bacteria and excreted into their surrounding environment. They are proteins in chemical composition and are relatively specific in terms of damage to the host.
- Endotoxins – are complex polysaccharides cell wall components of certain kind of bacteria. They are not released until the cell disintegrates. They are relatively heat stable, less specific in their actions and less potent than exotoxins.

The severity and distribution of any infectious disease are influenced by the manner in which the agent, the host and factors within the environment interact.

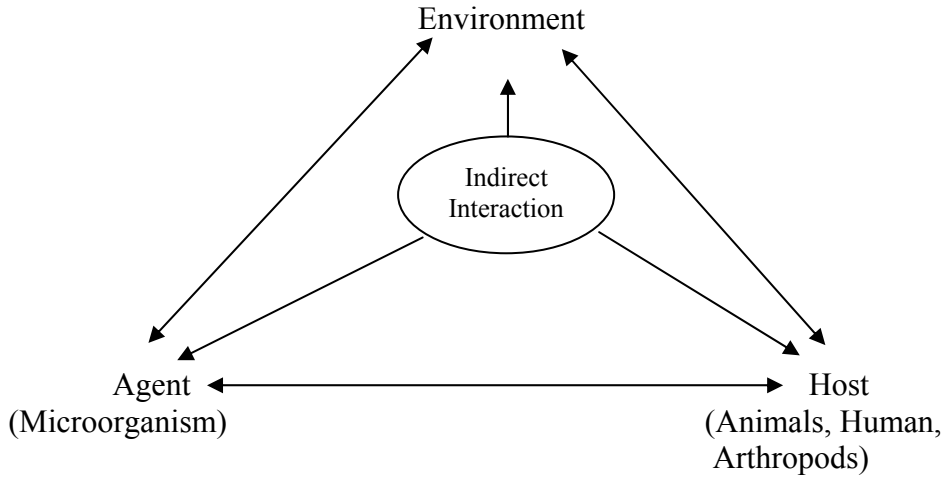

Figure 9.3: Generalized version of the triangle of causation for any kind of communicable disease

The main causes of reported food borne diseases are due to foods being mishandled. Foods that are implicated are usually "potentially hazardous" which are capable of supportive growth of disease-causing microorganisms. The various factors leading to FBD are listed in Table.

Table 9.1: Factors leading to reported food borne diseases (Ranked by % number of outbreaks)

40%	Improper cooling of foods
21%	Time lapse between preparing to serving
20%	Infected persons touching foods
16%	Inadequate cooling
16%	Improper hot storage
12%	Inadequate reheating
11%	Contaminated raw food
7%	Cross-contamination
7%	Improper cleaning
4%	Use of leftovers

9.4 CHEMICAL CONTAMINANTS OF FOOD

Poisoning by consumption of chemicals is rather uncommon and usually is characterized by appearance of the symptoms within a short period of time after the poisonous food is eaten. Various chemical contaminants include:

i) Fumigants are used to sterilize food under conditions in which steam heating is impractical. Ethylene oxide is a commonly used fumigant, which reacts with food constituents and destroys essential nutrients. It reacts with inorganic chloride to form ethylene chloro hydrine, which is toxic.

ii) Various solvents are used for the extraction of oil from oilseeds. But solvents like trichloro ethylene react with the foodstuff being processed with the formation of toxic products.

iii) Smoking of meat and fish for preservation and flavouring is an old practice. This processing contaminates the food with polycyclic hydrocarbons such as benzopyrene, many of which are carcinogenic.

iv) Metals are one of the many unintentional contaminants of food. When present beyond small quantities they are toxic. They find their way into food through air, water, soil, industrial pollution and other routes. Antimony, arsenic, cadmium, chlorinated hydrocarbons, copper, cyanide, fluoride, lead, selenium, mercury and zinc in foods have been blamed for food poisoning. Poisonous chemicals may enter foods from utensils e.g. from cheap enamelled utensils which contain antimony. Lead and arsenic residues from fruit sprays maybe on the surface of fruits but usually in harmless amounts, especially after washing. Symptoms of lead poisoning include weakness, dental caries, nausea, pains and paralysis. A major source of tin contamination is tin plate, which is used for containers of all types of processed foods. Canned foods if acidic and foods stored in tins after opening, change in colour, or develop a metallic flavour that is unacceptable. Insecticides, pesticides, growth regulators, fungicides and growth stimulators are essential in modern agriculture for the production of adequate quantities of sound food. They include insecticides like lead arsenate, organophosphate compounds (malathion), dinitro compounds. Toxicity levels for human vary.

Check Your Progress Exercise 1

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

1. What are food borne diseases?

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2. Differentiate between exotoxins and endotoxins.

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3. List the various chemical contaminants in food.

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Table 9.2: Toxicity of some metals in different foods

Metal	Type of Food	Toxic Effect
Arsenic	Fruits sprayed with lead arsenate	Dizziness, chills, cramps, paralysis leading to death
Barium	Foods contaminated with rat poison (barium carbonate)	Violet peristalsis, muscular twitching and convulsions
Cadmium	Fruit juice, soft drinks in contact with cadmium plated vessels	Excessive salivation and kidney damage, prostrate cancer, multiple fractures
Cobalt	Water, beer	Cardiac failure
Copper	Acid foods in contact with tarnished copper ware and brass utensils	Vomiting, diarrhea, abdominal pain
Lead	Processed foods	Paralysis, brain damage
Mercury	Mercury fungicide treated seed grains or mercury contaminated fish	Paralysis, brain damage and blindness
Tin	Canned foods	Colic, vomiting. Photophobia
Zinc	Foods stored in galvanized iron ware	Dizziness, vomiting

Table 9.3: Toxicity in food due to various pesticides and chemicals

Name of Pesticide	Type of Food	Toxic Effect
Pesticides	All type of raw, cooked, processed, canned foods	Acute or chronic poisoning causing damage to liver, kidney, brain, nerves leading to death
Diethylstil bestrol	Meat of still bestrol fed animals	Teratogenesis, carcinogenesis
Antibiotics	Meat of animals fed antibiotics	Drug resistance, hardening of arteries, heart diseases

Table 9.4: Permissible limits of some metals in foods

Name of metal	Foodstuff	PPM
Arsenic	1. Milk	0.10
	2. Beverages	0.50
	3. Soft Drinks	0.50
	4. Ice creams and frozen confections	0.50
	5. Dehydrated onions	2.0
	6. Dried spices and herbs	5.0
Copper	1. Beverages, soft drinks	7.0
	2. Tomato ketchup	50.0
	3. Cocoa powder	70.0
	4. Tomato puree, paste, juice powder	100.0
	5. Sugar confectionary	5.0
Lead	1. Beverages and soft drinks	0.50
	2. Fruits and vegetable juices	1.0
	3. Ice cream, frozen confections, canned fish, meat, dehydrated vegetables	1.0
Tin	1. Processed and canned foods	5.0
	2. Hard boiled sugar confectionary	5.0
Zinc	1. Beverages	5.0
	2. Fruit products	50.0
	3. Hard boiled sugar confectionary	5.0

9.5 NON-BACTERIAL MICROBIOLOGICAL CONTAMINATION OF FOOD

Microbiological contamination of food may include food borne illness due to bacteria or non bacterial sources such as mycotoxins, viruses, rickettsias, parasitic worms or protozoa or from the consumption of food contaminated with toxic substances. The food borne infections caused by bacteria will be dealt with in Unit 3 in detail.

9.5.1 Viruses

Much less is known about the incidence of viruses in foods than about bacteria and fungi because they do not grow in culture media as do bacteria and fungi, they do not replicate in foods thus found in low numbers. It is noted that virtually any food can serve as a vehicle for virus transmission. The most common food source of gastroenteritis causing virus is shellfish.

Hepatitis A virus

There are more documented outbreaks of hepatitis A traced to foods than any other viral infection. The virus causes hepatitis (jaundice) and leads to inflammation of liver. The incubation period for infectious hepatitis ranges from 15 to 45 days and lifetime immunity usually occurs after an attack. The fecal-oral route is the mode of transmission, and raw or partially cooked shellfish from polluted waters is the most common vehicle food. Shellfish are able to concentrate the numbers of bacteria or viruses during their normal feeding, which is to filter and remove particles from the water. The infectious hepatitis virus has been shown to be stable during refrigerated storage of shellfish. In addition to shellfish, raw milk, potato salad, sandwiches and cold meat cuts are also probable sources of the virus. Symptoms of jaundice include loss of appetite, yellowing of eyes, nails and skin (due to presence of bile pigments) and gastrointestinal disorder. Proper cooking, hygiene, sanitation and personnel cleanliness help to prevent virus attack.

Polio Virus

There are a large number of reported food-borne outbreaks of polio in India. It is most common in children up to 5 years. Milk is the most probable food there causes spread of polio virus. The virus reproduces in the intestinal tract, from there it invades the motor cells of the central nervous system. Initial symptoms are gastrointestinal, headache, muscle pain and paralysis. The paralytic symptoms range from sub clinical to fatal. Preventive measures include immunization of children, proper processing (pasteurization) of milk, hygienic conditions and use of potable water.

Norwalk Virus and Norwalk-like Viruses

These viruses, also known as small round structured viruses or caliciviruses, are an important cause of gastrointestinal illness throughout the United States. Members of this category of viruses are typically named for the location in which they were first identified, for example, Hawaii, Snow Mountain, Montgomery County and Oklahoma. The Norwalk virus is the prototype for this group of viruses – there are at least 11 other related viruses – hence the name "Norwalk-like virus."

Symptoms: The signs and symptoms of Norwalk-like viruses include nausea, vomiting, diarrhea, abdominal pain, muscle aches, headache, tiredness and low-grade fever. Symptoms typically last 24 hours to 48 hours and subside on their own. There are no known long-term effects after recovery from this infection.

Transmission of viruses: Humans are the only source for these viruses. These viruses do not multiply outside the human body. The viruses are present in the feces of infected persons and can be transmitted to others when hands are not thoroughly washed after having a bowel movement.

9.5.2 Rickettsias

Rickettsias maybe considered as degenerative bacteria since they represent a form of life closely resembling bacteria except that they cannot be cultivated outside of living cells. Like the viruses they are obligate parasites. Many of the major human rickettsial diseases are by bites from fleas, lice or ticks. Examples of human rickettsial diseases include epidemic typhus, rickettsial

pox, Rocky mountain spotted fever and Q fever. Cows infected with rickettsia of Q fever, *Coxiella brunettii* excrete contaminated milk which result in human infections. Hence milk is pasteurized at a minimum temperature of 62.8ºC for 30 minutes to ensure its destruction.

9.5.3 Food Borne Parasites

Trichinosis

Trichinella spiralis causes trichinosis, which results from the consumption of raw or incompletely cooked pork containing the encysted larvae.

Symptoms: One or two days after ingestion of heavily encysted meat, trichinae penetrate the intestinal mucosa, producing nausea, abdominal pain, diarrhea and sometimes vomiting. The symptoms may persist for several days. The larvae then attack the skeletal muscles, muscle pain (paralysis) is the universal symptom accompanied in difficulty in breathing, chewing and swallowing. After six months of initial infection, pain, swelling and fever occur.

Prevention and control: Chief method for prevention of trichinosis is the treatment of pork (or other meat) to ensure the destruction of any trichinae that maybe present by cooking of pork till at least 58.3ºC, quick freezing or storage at -15ºC or lower for not less than 20 days, irradiating or processing of sausage and similar meat products properly by salting, drying, smoking and refrigeration. Also trichinosis can be controlled by avoiding feeding of infected meat scraps to swine and by preventing the consumption of infested tissue by other animals.

Amoebiasis

Entamoeba histolytica is a single celled parasitic animal – a protozoa, that infects predominantly humans and other primates and causes amoebiasis. Diverse mammals such as dogs and cats can become infected but usually do not shed cysts (the environmental survival form of the organism) with their feces, thus do not contribute significantly to transmission. The active (trophozoite) stage exists only in the host and in fresh feces; cysts survive outside the host in water and soil and on foods, especially under moist conditions on the latter. When swallowed they cause infections by excysting (to the trophozoite stage) in the digestive tract (amoebiasis).

Symptoms: Infections that sometimes last for years may be accompanied by 1) no symptoms, 2) vague gastrointestinal distress, 3) dysentery (with blood and mucus). Most infections occur in the digestive tract but other tissues may be invaded. Complications include 4) ulcerative and abscess pain and, rarely, 5) intestinal blockage. Onset time is highly variable. It is theorized that the absence of symptoms or their intensity varies with such factors as 1) strain of amoeba, 2) immune health of the host, and 3) associated bacteria and, perhaps, viruses. The amoeba's enzymes help it to penetrate and digest human tissues; it secretes toxic substances.

Diagnosis: The ingestion of one viable cyst can cause an infection. Human cases are diagnosed by finding cysts shed with the stool; various flotation or sedimentation procedures have been developed to recover the cysts from fecal matter; stains (including fluorescent antibody) help to visualize the isolated cysts for microscopic examination. Since cysts are not shed constantly, a

minimum of 3 stools should be examined. In heavy infections, the motile form (the trophozoite) can be seen in fresh feces. Serological tests exist for long-term infections. It is important to distinguish the *E. histolytica* cyst from the cysts of nonpathogenic intestinal protozoa by its appearance.

Transmission: Amebiasis is transmitted by fecal contamination of drinking water and foods, but also by direct contact with dirty hands or objects as well as by sexual contact.

Giardiasis

Giardia lamblia (intestinalis) is a single celled animal, i.e., a protozoa, that moves with the aid of five flagella. Organisms that appear identical to those that cause human illness have been isolated from domestic animals (dogs and cats) and wild animals (beavers and bears). A related but morphologically distinct organism infects rodents, although rodents may be infected with human isolates in the laboratory. Human giardiasis may involve diarrhea within 1 week of ingestion of the cyst, which is the environmental survival form and infective stage of the organism.

Symptoms: Normally illness lasts for 1 to 2 weeks, but there are cases of chronic infections lasting months to years. Chronic cases, both those with defined immune deficiencies and those without, are difficult to treat. The disease mechanism is unknown, with some investigators reporting that the organism produces a toxin while others are unable to confirm its existence. The organism has been demonstrated inside host cells in the duodenum, but most investigators think this is such an infrequent occurrence that it is not responsible for disease symptoms. Mechanical obstruction of the absorptive surface of the intestine has been proposed as a possible pathogenic mechanism, as has a synergistic relationship with some of the intestinal flora.

Diagnosis: *Giardia lamblia* is frequently diagnosed by visualizing the organism, either the trophozoite (active reproducing form) or the cyst (the resting stage that is resistant to adverse environmental conditions) in stained preparations or unstained wet mounts with the aid of a microscope. A commercial fluorescent antibody kit is available to stain the organism. Organisms may be concentrated by sedimentation or flotation; however, these procedures reduce the number of recognizable organisms in the sample.

Associated foods: Giardiasis is most frequently associated with the consumption of contaminated water. Outbreaks have been traced to food contamination by infected or infested food handlers, and the possibility of infections from contaminated vegetables that are eaten raw cannot be excluded. Cool moist conditions favor the survival of the organism.

Prevalence: Giardiasis is more prevalent in children than in adults, possibly because many individuals seem to have a lasting immunity after infection. This organism is implicated in 25% of the cases of gastrointestinal disease and may be present asymptomatically. This disease afflicts many homosexual men, both HIV-positive and HIV-negative individuals. This is presumed to be due to sexual transmission. The disease is also common in child day care centers, especially those in which diapering is done.

Ascariasis

Humans worldwide are infected with *Ascaris lumbricoides* and *Trichuris trichiura*; the eggs of these roundworms (nematode) which are "sticky" and may be carried to the mouth by hands, other body parts, fomites (inanimate objects), or foods. Ascariasis and trichuriasis are the scientific names of these infections. Ascariasis is also known commonly as the “large roundworm” infection and trichuriasis as “whip worm” infection.

Diagnosis: Infection with one or a few Ascaris sp. may be unapparent unless noticed when passed in the feces, or, on occasion, crawling up into the throat and trying to exit through the mouth or nose. Infection with numerous worms may result in a pneumonitis in the lungs, where the larvae break out of the pulmonary capillaries into the air sacs, ascend into the throat and descend to the small intestine again where they grow, becoming as large as 31 × 4 cm. Vague digestive tract discomfort sometimes accompanies the intestinal infection, but in small children with more than a few worms there may be intestinal blockage because of the worms' large size. Not all larval or adult worms stay on the path that is optimal for their development; those that wander may locate in diverse sites throughout the body and cause complications. Both infections are diagnosed by finding the typical eggs in the patient's feces; on occasion the larval or adult worms are found in the feces or, especially for *Ascaris* sp., in the throat, mouth, or nose.

Associated foods: The eggs of these worms are found in insufficiently treated sewage-fertilizer and in soils where they embryonate (i.e., larvae develop in fertilized eggs). The eggs may contaminate crops grown in soil or fertilized with sewage that has received nonlethal treatment; humans are infected when such produce is consumed raw. Infected food handlers may contaminate a wide variety of foods.

Prevention: Both infections may self-cure after the larvae have matured into adults or may require anthelmintic treatment. In severe cases, surgical removal may be necessary. Allergic symptoms (especially but not exclusively of the asthmatic sort) are common in long-lasting infections or upon reinfection in ascariasis.

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 are the non-bacterial food borne disease causing agents?

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2. What is the causative organism for jaundice and how is it transmitted?

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3. List the food-borne parasites. What are the symptoms of the disease cause by them?

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9.6 INVESTIGATION OF FOOD BORNE DISEASE OUTBREAK

Analysis of an outbreak involves field analysis as well as laboratory analysis and is undertaken by Ministry of Health. From public health point of view the objectives of the investigation of outbreak are:

1. *Prevention and control*

   Identification of contaminated foods

2. *Knowledge of disease causation*

   Observe the track record of various illnesses-causing agents

3. *Administration guidance*

   Assessment of trends to justify regulations, decisions/actions

This requires the location and the identification of the causative agent, establishing of the means of transmission, demonstration of opportunity for growth of the pathogen.

Personnel Involved in Investigation

The team to investigate an outbreak of food borne disease consists of a person in charge, a field group and a laboratory group. The field group interviews persons, both ill and healthy, who consumed the suspected foods, physicians and nurses who are treating the victims, and personnel at the place of exposure to the disease; collects samples of suspected foods and transits them to the laboratory, collects specimens from patients or food handlers when such sample is indicated; inspects the premises where the foods were stored,

prepared and served; fills out appropriate reports on these activities, and makes it available for laboratory staff. The laboratory group makes microbiological and chemical tests as indicated by reports of the field group and the nature of the suspected food and records its findings on appropriate report blanks. The person in-charge or a qualified epidemiologist then can interpret the data from all sources to determine the cause and source of disease outbreak.

Steps of Investigation

Field Analysis

a) Gathering information

The field group inspects the place or places where the suspected meal, meals or beverages were prepared and consumed and then record the results in appropriate forms. Information sought includes the menu for meals, source and method of preparation of each item on the menu, methods of storage of perishable foods, health of employees serving or preparing foods and their health history. The information obtained is recorded on a form entitled "Case History Questionnaire" as given.

Performa

Name of Person affected	Age	Time of First Symptom	Foods Consumed

The Performa indicate the most likely age group affected and the type of food being consumed by them. Also we get an idea of the type of food through which the infection could have probably been caused.

b) Sample collection

Samples of left over food and /or beverage served at suspected meal are aseptically collected by means of sterile sampling devices in sterile containers, labelled and transited to laboratory under refrigerated conditions.

c) Collection of specimens from human sources

Specimens maybe obtained from patients with food illnesses or from food handlers to ascertain the ultimate source of the pathogen that entered the food. Culture from nose, throat or skin lesions, fecal or blood samples may be done.

Laboratory Analysis

The sample received from the field are analyzed microbiologically and sometimes for chemical contamination also. The report is then sent to the in-charge, the epidemiologist (who is expert in dealing with epidemics).

Performa for Laboratory Report

Etiological Agent (Causative Agent of Spoilage)	Clinical Symptoms (of affected people)	Laboratory Investigation	Complete Investigation (Food responsible for Outbreak)	Preventive Measures
Eg: For *Bacillus gastroenteritis*		Investigation of food, stools and faeces. Isolation of the microorganism	Infection due to accidental contamination or otherwise	Asepsis, prevent inadequate treatments, proper storage conditions

Interpretation of Report

This is done by the epidemiologist and conclusion is taken out for cause of the outbreak. Complete investigation of food borne disease outbreak is given as:

i) Name of the reporting officer

ii) Name of the local authority who is analyzing the complete outbreak

iii) Area/place of outbreak

iv) Date and time when suspected meal was taken

v) Number of persons' affected

vi) Number of people at risk indicating those who have developed symptoms)

vii) Incubation Period

viii) Symptoms

ix) Occupational/age group

x) Details of suspected meal (complete analysis of food)

xi) Foods which are eaten by affected persons

xii) Number of meal sittings and time

xiii) Methods of cooking

xiv) Time and temperature of storage of cooking

xv) General notes regarding the facilities and equipments

Minimum Infrastructure/ Materials Required

- Field Kit for field analysts to collect sample, medical equipment, sterile containers, sampling device, sterilized thermometer, lamps of alcohol, sterile wrapping foil, tapes for sealing, sterile paper, towels, ice boxes, insulated boxes for carrying samples.
- Laboratory- Facility to find the total plate count and types of microorganisms, glassware, pipettes, flask, media, laminar flow, stains. Chemicals, specific test kits for enumerating specific organisms.
- Data interpretation infrastructure.

WHO'S Golden Rule for Safe Food Production

1. Choose foods processed for safety.
2. Cook foods thoroughly.

3. Eat cooked foods immediately.
4. Store cooked foods carefully.
5. Reheat cooked foods thoroughly.
6. Avoid contact between raw and cooked foods.
7. Wash hands repeatedly.
8. Keep all kitchen surfaces meticulously clean.
9. Protect foods from insects, rodents and other animals.
10. Use pure water.

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 are the main objectives for which investigation of any outbreak is done?

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2. Explain the various steps involved to carry out any investigation.

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3. State WHO's Golden rule for safe food production.

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9.7 LET US SUM UP

In this unit on food borne disease, we have examined what are the food borne disease and how to differentiate with food borne infections and intoxications. This includes study of various contaminants of food, namely, bacterial, non bacterial and toxic chemicals. We have also studied the importance of the investigation of food borne disease outbreak. The steps involved in the investigation were also studied. We have seen that food borne diseases maybe caused due to contaminated food and beverages. They are a common, distressing and sometimes life-threatening problem for millions of people all around the world. We also studied in the end the related golden rules for the prevention of such food borne disease outbreaks. Hence following good manufacturing practices (GMP's) and giving publicity to an outbreak and the explanation of its cause maybe helpful in educating and warning the public and avoiding further outbreaks.

9.8 KEY WORDS

Food Borne Disease : Disease caused by the consumption of contaminated foods or beverages

Food Borne Intoxication : Food borne disease caused due to ingestion of toxin already present in food.

Food Borne Infection : Food borne disease caused due to ingestion of causative organisms into the body where they multiply, grow and cause disease.

Exotoxin : Proteinaceous toxin produced by bacteria and excreted into surrounding environment

Endotoxin : Toxin produced by bacteria and excreted into surrounding environment after the cell lysis. Are mainly composed of polysaccharides.

9.9 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Food borne diseases are caused by consuming contaminated foods or beverages e.g., food borne infection and food borne intoxication.

2. Exotoxins are proteinaceous toxins produced by bacteria and excreted into surrounding environment.

   Endotoxins are toxins produced by bacteria and excreted into surrounding environment after the cell lysis (breaking of cell membrane), which are mainly composed of polysaccharides.

3. Chemical contaminants in food include fumigants, solvents like hexane, metallic contaminants such as antimony, arsenic, cadmium etc., insecticides, pesticides and growth regulators etc.

Check Your Progress Exercise 2

1. Non-bacterial food borne disease causing agents include viruses, rickettsias and parasites (*Trichinella, Entamoeba, Giardia, Ascaris*)

2. Jaundice is caused by Hepatitis A virus. through faecal-oral transmission. Foods associated are raw or partially cooked shellfish, raw milk, potato sandwiches and cold meat cuts.

3. Food borne parasites include:

   a) *Trichinells spiralis* (causes Trichinosis) characterized by nausea, abdominal pain, diarrhea and vomiting. Severe attack may cause paralysis, fever and severe pain.

   b) *Entamoeba histolytica* (causes amoebiasis) results in gastrointestinal distress, dysentery, ulcers and intestinal blockage.

   c) *Giardia lamblia* (causes giardiasis) results in severe gastroenteritis and intestinal problems.

   d) *Ascaris lumbricoides* causes ascariasis, resulting in digestive tract disturbance, intestinal disorder and blockage.

Check Your Progress Exercise 3

1. Objectives of food borne disease outbreak investigation are:

   - To prevent and control contamination of foods.
   - To know the disease causing agents.
   - To develop guidelines and take regulatory decisions.

2. Three steps in the investigation of any food borne disease outbreak:

   - **Field Analysis:** Collection of samples and data pertaining to the causes of outbreak in the particular area.

   - **Laboratory Analysis** dealing with the isolation of the causative organism from the various collected samples.

   - **Identification and the Interpretation of Report** involving the compilation of all facts and figures and stating the cause of outbreak and the possible causative measures to be taken.

3. WHO's Golden rule for safe food production states that:

   - Choose foods processed for safety
   - Cook foods thoroughly
   - Eat cooked foods immediately
   - Store cooked foods carefully
   - Reheat cooked foods thoroughly
   - Avoid contact between raw and cooked foods
   - Wash hands repeatedly
   - Keep all kitchen surfaces meticulously clean
   - Protect foods from insects, rodents and other animals
   - Use pure water

9.10 SOME USEFUL BOOKS

1. A manual entitled 'Procedures to Investigate Food borne Illnesses' (1976) has been written by the Committee on communicable Diseases Affecting Man in the International Association of Milk, Food and Environmental Sanitarians and provides excellent information on such food borne diseases and their outbreaks. A complete listing of diseases transmitted by foods, including etiologic agents, nature of the organism, incubation period, signs and symptoms, source or reservoir, epidemiology, foods involved and control measures can be found in Center for Disease Control (1976a).

2. Frazier, W.C. and Westoff, D.C. (1988) Food Microbiology, Tata McGraw-Hill Pub. Co., New Delhi. pp. 539.

3. Purohit, S.S. (1994) Microbiology-Fundamental and Application. 5th edn. Agro Botanical Publishers, Bikaner, India.

4. Srivastava, R.P. and Kumar, S. (1994) Fruits and Vegetable Preservation, International Book Distribution Co., Lucknow.

UNIT 12 CHEMICAL

Structure

12.0 OBJECTIVES

After studying this unit, you should be able to:

- know about different types of chemical preservatives; and
- define the essential chemical preservative limits for the various foods.

12.1 INTRODUCTION

In this unit we will make you aware about the characteristics of various chemical preservatives with special stress on antimicrobial food preservatives. We will also brief you about different chemical preservatives permitted in processed products along with maximum levels of antimicrobials permitted in foods. You will also learn about various factors which determine/ influence the action of chemical food preservatives.

12.1.1 Need for Food Preservation

Unless you grow all your food in your own garden and prepare all your meals from scratch, it's almost impossible to eat food without preservatives added by manufacturers during processing.

Food preservation is a method of preparing food so that it can be stored for future use. Because most foods remain edible for only a brief period of time, people since the earliest ages have experimented with methods for successful food preservation. Among the products of early food conservation were cheese and butter, raisins, pemmican, sausage, bacon, and grain. Scientific investigations pointed that food spoilage was mainly caused by microorganisms widely distributed in the environment. Therefore, food

preservation depends on rendering conditions unfavourable for microbial growth

12.1.2 Techniques of Food Preservation

The techniques of food preservation can be separated into two groups:

- physical
- chemical

Physical methods of preservation rely on killing the microorganisms present, or at least stopping their growth for long enough to allow the food to be safely consumed. The physical methods include canning, freezing, drying, gamma irradiation, ultraviolet or high intensity white light, ultra high pressure and filtration.

Chemical food preservatives are substances which, under certain conditions, either delay the growth of microorganisms without necessarily destroying them. These are added in very low quantities and which do not alter the organoleptic and physico-chemical properties of the foods at or only very little. These work either as direct microbial poisons or by reducing the pH to a level of acidity that prevents the growth of microorganisms.

12.2 CHARACTERISTICS OF CHEMICAL PRESERVATIVES

The Food, Drug, and Cosmetic Act permit for the use of chemical preservatives in foods if the chemical is:

1. Generally recognized as safe (GRAS) for such use; or if a food additive is covered by food additive regulations prescribing conditions of safe use.
2. Not used in such a way as to conceal damage or inferiority or to make the food appear better or of greater value than it is.
3. Properly declared on the label of the food in which used.
4. It should be food grade.
5. It should perform its intended function.
6. It should be used in accordance with good manufacturing practices and, where applicable, in accord with existing food additive regulations.

According to rules, a food manufacturer must get approval from Government regulatory authorities before using a new preservative, or before using a previously approved preservative in a new way or in a different amount. In its petition for approval, the manufacturer must demonstrate that the preservative is safe for consumers, considering:

- the probable amount of the preservative that will be consumed with the food product, or the amount of any substance formed in or on the food resulting from use of the preservative
- the cumulative effect of the preservative in the diet
- the potential toxicity (including cancer-causing) of the preservative when ingested by humans or animals.

- A preservative may not be used to deceive a consumer by changing the food to make it appear other than it is. For example, preservatives that contain sulfites are prohibited on meats because they restore the red colour, giving meat a false appearance of freshness.
- The food additive regulations require the preservative to be of food grade and be prepared and handled as a food ingredient.
- The quantity added to food must not exceed the amount needed to achieve the manufacturer's intended effect.

Regulations about the use of nitrites demonstrate the scrutiny given to the use of additives. Nitrites, used in combination with salt, serve as antimicrobials in meat to inhibit the growth of bacterial spores that cause botulism, a deadly food-borne illness. Nitrites are also used as preservatives and for flavouring and fixing colour in a number of red meat, poultry, and fish products. Since the original approvals were granted for specific uses of sodium nitrite, safety concerns have arisen. Nitrite salts can react with certain amines (derivatives of ammonia) in food to produce nitrosamines, many of which are known to cause cancer. A food manufacturer wanting to use sodium nitrites must show that nitrosamines will not form in hazardous amounts in the product under the additive's intended conditions of use. For example, regulations specify that sodium nitrite, used as an antimicrobial against the formation of botulinum toxin in smoked fish, must be present in 100 to 200 parts per million. In addition, other antioxidants, such as sodium ascorbate or sodium erythorbate, may be added to inhibit the formation of nitrosamines.

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 chemical preservatives?

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2. Name three important characteristics of chemical preservatives?

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3. Name important facts that a manufacturer must demonstrate to regulatory authorities for getting a new preservative formulation approved?

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12.3 CLASSIFICATION OF PRESERVATIVES

Preservatives can be categorized into following types:

1. Antimicrobials that inhibit growth of bacteria, yeasts, or molds.
2. Antioxidants that slow air oxidation of fats and lipids, which leads to rancidity.
3. Antienzymatic that blocks the natural ripening and enzymatic processes that continue to occur in foodstuffs after harvest.
4. Preservatives from natural products.
5. Traditional preservatives

We will discuss the first type of preservatives in detail but before that brief description of other types is given below for the sake of awareness.

12.3.1 Antioxidant Preservatives

As antioxidants, they keep foods from becoming rancid, browning, or developing black spots. Rancid foods may not make you sick, but they smell and taste bad. Antioxidants suppress the reaction that occurs when foods combine with oxygen in the presence of light, heat, and some metals. Antioxidants also minimize the damage to some essential amino acids--the building blocks of proteins--and the loss of some vitamins.

Antioxidant preservatives, such as butylated hydroxytoluene, butylated hydroxyanisole, *tert*-butylhydroquinone, and propyl gallate, stop the chemical breakdown of food that happens in the presence of oxygen. Unsaturated fatty acids in oils and lipids are particularly susceptible to autooxidation. In this process, a free radical initiates peroxide formation at fatty acid double bonds. The chain reaction propagates to other double bonds, and aldehyde, ketone, and acid-termination products eventually build up to create the rancid off-flavors characteristic of oils and fats gone bad. Antioxidant preservatives sop up the free radicals that help initiate and propagate these reactions.

12.3.2 Preservatives that Targets Enzymes

These are preservatives that target enzymes in the food itself that continue to metabolize after harvest. The enzyme phenolase, for example, goes to work as soon as an apple or potato is cut. It browns the exposed surface. Acids such as citric acid and ascorbic acid (vitamin C) inhibit phenolase by making the pH

uncomfortably low for the enzyme. Metal-chelating agents such as EDTA (ethylenediamine tetraacetic acid) can remove the metal cofactors that many enzymes need. Chelators also make it difficult for bacterial and fungal enzymes to carry on.

12.3.3 Preservatives from Natural Products

Some of the newest antimicrobials have been found in microorganisms themselves as they form their own chemical defenses when competing with each other for space and nutrients. For example, nisin and natamycin, the cheese preservatives called bacteriocins – are harvested from microorganisms. In the U.S., nisin is used to inhibit outgrowth of *Clostridium botulinum* spores (the cause of botulism) and toxin formation in pasteurized process cheese spreads with fruits, vegetables or meats at levels not exceeding good manufacturing practice. Current good manufacturing practice in this case is the quantity of the ingredient that delivers a maximum of 250 p.p.m. of nisin in the finished product. Nisaplin-brand nisin is also approved for liquid egg products, dressings, and sauces. In other countries it is also used in fresh and recombined milk, fermented beverages like beer, canned foods, frozen desserts, and high moisture/reduced fat foods. Nisin is considered effective at controlling a wide range of gram-positive organisms including: *Listeria enterococcus*, *Bacillus sporothermodurans*, and *clostridium*. Used alone, it is not effective on gram-negative bacteria (like *E. coli*), yeasts, and molds. However, research suggests that it may be useful against some gram-negative bacteria when used in conjunction with other preservatives.

12.3.4 Traditional Chemical Food Preservatives

Traditional chemical food preservatives and their use in fruit and vegetable processing technologies are common salt and sugar.

Common salt used in brined vegetables. There is no limit for their use.

Sugars (sucrose, glucose, fructose and syrups): foods preserved by high sugar concentrations such as jellies, preserves, syrups, juice concentrates. It acts by interaction of sugar with other ingredients or processes such as drying and heating. There is no limit for their use.

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. Name two anti-oxidant preservatives?

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2. How does ascorbic and inhibit phenolase enzyme?

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3. Name two preservatives of microbial origin?

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4. Is there any limit for traditional food preservatives?

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12.4 ANTIMICROBIAL PRESERVATIVES

Usually accepted chemical food preservatives are detailed in Table 12.1.

Table 12.1: Commonly used antimicrobial chemical food preservatives

Agent	Acceptable Daily intake (mg/Kg body weight)	Commonly used levels (%)	Typical usage
Sorbic acid Potassium sorbate	25	0.05-0.2	fruits; vegetables; pickled products; jams, jellies
Benzoic acid Sodium benzoate	5	0.03-0.2	Vegetable pickles; preserves; jams; jellies; semi-processed products
Propionic acid Sodium propionate	10	0.1-0.3	Bakery goods, cheese spread, fruits, vegetables
Methyl paraben Ethyl paraben Propyl paraben	10	0.05-0.1	Bakery goods, fruit products; pickles; sauces
Lactic acid	No limit	No limit	Fermented meat, dairy and vegetable products, sauces and dressings, drinks.
Citric acid	No limit	No limit	fruit juices; jams; other sugar preserves
Acetic acid	No limit	No limit	vegetable pickles; other vegetable sauces, chutney
Sodium nitrite	0.2	0.01-0.02	Meat products
Sulphur dioxide	0.7	0.005-0.2	fruit juices, dried / dehydrated fruits and vegetables, semi-processed products

12.4.1 Organic Acids and Esters

Sodium Benzoate and Benzoic Acid

Benzoic acid is the compound with the antimicrobial properties, and is found naturally in cranberries, prunes, greengage plums, cinnamon, ripe cloves and apples. Sodium benzoate produces benzoic acid once it is dissolved in water.

Sodium benzoate is the sodium salt of benzoic acid and is preferred over benzoic acid in many food applications because it is 180 times more soluble in water. There is a marked pH effect for this preservative: the lower the pH, the more effective it is. Sodium benzoate will only work if the food product has a pH below 4.5; that is, if the food is naturally acidic or has been acidified.

For example, at pH 3.0 you only need approximately 0.05% of the compound to achieve the same antimicrobial effect as pH 4.0 and 0.1% benzoate. Optimum functionality occurs when the pH is between 2.5 and 4.0.

Sodium benzoate is used in fruit products, jams, relishes, beverages, dressings, salads, pie and pastry fillings, icings, olives and sauerkraut, and is effective against yeasts, some bacteria (food borne pathogens but not spoilage bacteria) and some molds. Sodium benzoate is a white granular or crystalline powder, odorless, inexpensive (at the usage level) and should be stored in a cool, dry place in watertight containers, if possible. It should be used at low levels to avoid possible off-flavours in some products. The maximum level allowable by law is 0.1%.

Sorbates

This family of compounds are available as sorbic acid, potassium sorbate, sodium sorbate or calcium sorbate. Sorbic acid is the compound with the antimicrobial properties but its salts (sorbates) are used in many cases due to differences in solubility.

Potassium sorbate is the potassium salt of sorbic acid, and is much more soluble in water than the acid. It is a white crystalline powder, inexpensive (at the usage level), with basically no noticeable flavour at normal usage concentrations. In wine processing, sorbates are used to prevent refermentation. Maximum level allowable by law is 0.1%. It produces sorbic acid once it is dissolved in water and is the most widely used food preservative in the world. It is effective up to pH 6.5 but effectiveness increases as the pH decreases. It has about 74% of the antimicrobial activity of the sorbic acid, thus requiring higher concentrations to obtain the same results that pure sorbic acid provides. It is effective against yeasts, molds, and select bacteria, and is widely used at 0.025 to 0.10 % levels in cheese, dips, yogurt, sour cream, bread, cakes, pies and fillings, baking mixes, doughs, icings, fudges, toppings, beverages, margarine, salads, fermented and acidified vegetables, olives, fruit products, dressings, smoked and salted fish, confections and mayonnaise.

It is important to know that the addition of sodium benzoate and/or potassium sorbate to a food product will raise the pH by approximately 0.1 to 0.5 pH units depending on the amount, pH, and type of product. Additional adjustment of the pH might be needed to keep the pH at a safe level.

In many food products, sorbate and benzoate are used together to provide greater protection against a wider variety of microorganisms. This only makes sense if the pH of the product is below 4.5.

Propionic acid

Propionic acid occurs naturally in strawberries, apples, violet leaves, grains. It is produced during the fermentation of some cheeses such as Swiss cheese, in concentrations as high as 1%, thus inhibiting the growth of molds. The acid is effective against bread molds and the spores of the bacterium *Bacillus mesentericus*, which cause an inedible condition in baked goods called rope. It is an oily liquid, soluble in water, with a slight pungent, disagreeable, rancid odour. It is also corrosive and flammable, thus requiring special handling.

Propionic acid and its salts, sodium and calcium propionates, are approved in the United States as GRAS (Generally Recognized As Safe) substances for

food use. Their antimicrobial action is directed to molds and rope bacteria, with almost no effect on yeast, thus making them an ideal choice for products that use commercial yeast as an ingredient.

Like other preservatives, propionates effectiveness is affected by the pH of the food, with 5.5 pH being the upper effective limit. They are used mainly as mold and rope inhibitors in bread; although they are also useful in cheese, non-alcoholic beverages, confections, fillings, frostings, fresh dough, pizza crust, puddings, gelatins, jams, jellies and some meat products.

The sodium and calcium salts are transparent and white crystals with a mild cheese like flavour. The sodium form is more soluble in water than the calcium salt. Sodium propionate is recommended in baked products that use baking powder and baking soda instead of yeast as the leavening agent, because the presence of calcium ions (if you were to use calcium propionate) disrupts the leavening process. Calcium propionate is preferred in baked foods that use yeast, such as breads and rolls, because the nutritional value is increased by the added calcium.

Typical usage level of propionic acid and propionates is 0.1 to 0.4 %. Federal regulations limit the maximum level for flour, white bread and rolls at 0.32% based on the weight of the flour; for whole wheat products at 0.38% based on the weight of the flour; and for cheese products at 0.3 %.

It is important to know that the addition of sodium and calcium propionate to a food product will raise the pH by approximately 0.1 to 0.5 pH units depending on the amount, pH and type of product. Additional adjustment of the pH might be needed to keep the pH at a safe level.

Parabens

The parabens are esters of para-hydroxibenzoic acid. The two most common esters are methyl and propyl parabens, which are approved for food use in the United States under the GRAS classification. The maximum concentration allowed is 0.1 %. They are most active against yeasts and molds.

Parabens are white powders with faint odour and fair solubility in water at room temperature. The solubility is greatly increased by heating the water to 71.1°C-82.2°C. Methyl paraben is more soluble in water but less effective against molds than propyl paraben. To balance these differences, mixtures of 2 to 3 parts of methyl paraben with 1 part propyl paraben are normally used.

Important advantages of parabens are their effectiveness at higher pH values, from 3 up to 8, and stability to high and low temperatures, even to steam sterilization. Despite these properties, parabens are not as widely used as other antimicrobial agents, probably due to higher cost and flavour objections. Applications include bakery products (formulated without yeast), beverages, flavour extracts, food colours, fruit products, jams, jellies, preserves (artificially sweetened), gelatin, marinated and smoked fish, pickles, salad dressings, syrups, wine and olives.

Lactic acid

This acid is the main product of many food fermentations; it is formed by microbial degradation of sugars in products such as sauerkraut and pickles. The acid produced in such fermentations decreases the pH to levels unfavourable for growth of spoilage organisms such as putrefactive anaerobes

and butyric-acid-producing bacteria. Yeasts and molds that can grow at such pH levels can be controlled by the inclusion of other preservatives such as sorbate and benzoate.

Acetic acid

Acetic acid is a general preservative inhibiting many species of bacteria, yeasts and to a lesser extent molds. It is also a product of the lactic-acid fermentation, and its preservative action even at identical pH levels is greater than that of lactic acid. The main applications of vinegar (acetic acid) includes products such as pickles, sauces and ketchup.

12.4.2 Gaseous Chemical Food Preservatives

Sulphur dioxide and sulphites

Sulphur dioxide (SO_2) has been used for many centuries as a fumigant and especially as a wine preservative. It is a colourless, suffocating, pungent-smelling, non-flammable gas and is very soluble in cold water (85 g in 100 ml at 25°C).

Sulphur dioxide and its various sulphites when dissolved in water at low pH yield sulphurous acid, bisulphite and sulphite ions. The various sulphite salts contain 50-68% active sulphur dioxide. A pH dependent equilibrium is formed in water and the proportion of SO_2 ions increases with decreasing pH values. At pH values less than 4.0 the antimicrobial activity reaches its maximum.

Sulphur dioxide is used as a gas or in the form of its sulphite, bisulphite and metabisulphite salts which are powders. The gaseous form is produced either by burning Sulphur or by its release from the compressed liquefied form.

Metabisulphite are more stable to oxidation than bisulphites, which in turn show greater stability than sulphites.

Mode of action

Sulphites inhibit microbial growth through a number of actions. They react with the energy rich compound, adenosine triphosphate; inhibit some metabolic pathways; and block cellular transport systems. Other antimicrobials alter microbial membrane or cell wall permeability or destroy the genetic material. In addition to its antimicrobial action, sulphur dioxide inhibits degradation reactions in fruits. It keeps raisins and other dried fruits from losing their light colour by blocking both enzymatic browning and a nonenzymatic browning reaction between reducing sugars and amino acids called the Maillard reaction. The reaction darkens raisins, alters their flavour, and reduces essential amino acid levels.

Uses

Sulphites are used to prevent or reduce discolouration of light-coloured fruits and vegetables, such as dried apples and dehydrated potatoes. These are added to sun-dried tomatoes, dried apricots, dried sweet potatoes, balsamic vinegar, red wine vinegar, lemon juice, and Hawaiian coconut syrup. These are also commonly used to lengthen the life of fruit juices. They are also used in wine-making because they inhibit bacterial growth but do not interfere with the desired development of yeast. Sulphites are also used in other ways, such as for bleaching food starches and as preventives against rust and scale in boiler

water used in making steam that will come in contact with food. Some sulphites are used in the production of cellophane for food packaging.

Precautions

FDA prohibits the use of sulphites in foods that are important sources of thiamin (vitamin B1), such as enriched flour, because sulphites destroy the nutrient. It causes severe allergic reactions, especially in asthmatics though, for the majority of the population, they are safe.

According to FDA sulphites used specifically as preservatives must be listed on the label, regardless of the amount in the finished product. Sulphites used in food processing but not serving as preservatives in the final food must be listed on the label if present at levels of 10 parts per million or higher.

According to FDA the use of sulphites on fruits and vegetables intended to be eaten raw is banned. These were used to maintain the colour and crispness of fresh produce.

Carbon dioxide (CO_2)

CO_2 is a colourless, odourless, non-combustible gas, acidic in odour and flavour. In commercial practice it is sold as a liquid under pressure (58 kg per cm^3) or solidified as dry ice.

Carbon dioxide is used as a solid (dry ice) in many countries as a means of low-temperature storage and transportation of food products. Beside keeping the temperature low, as it sublimes, the gaseous CO_2 inhibits growth of psychrotrophic microorganisms and prevents spoilage of the fruits and vegetables, etc.

It is used as a direct additive in the storage of fruits and vegetables. In the controlled/ modified environment storage of fruit and vegetables, the correct combination of O_2 and CO_2 delays respiration and ripening as well as retarding mold and yeast growth.

The final result is an extended storage of the products for transportation and for consumption during the off-season. The amount of CO_2 (5-10%) is determined by factors such as nature of product, variety, climate and extent of storage.

Chlorine

The various forms of chlorine constitute the most widely used chemical sanitizer in the food industry. These chlorine forms include chlorine (Cl_2), sodium hypochlorite (NaOCl), calcium hypochlorite ($Ca(OCl)_2$) and chlorine dioxide gas (ClO_2).These compounds are used as water adjuncts in processes such as product washing, transport, and cooling of heat-sterilised cans; in sanitising solutions for equipment surfaces, etc.

Important applications of chlorine and its compounds include disinfection of drinking water and sanitation of food processing equipment.

12.4.3 Nitrites and Nitrates

Nitrites and nitrates are used mainly among the packaged meats. Sodium nitrate is added to meats such as ham, bacon, hot dogs and smoked fish. Nitrates break down in the body to nitrites and this stops the growth of bacteria, especially the bacteria that cause botulism poisoning. They are the

food industry's primary chemical defense against the bacterium *Clostridium botulinum*.

It also stabilizes the red colour in cured meat and stops it turning grey. Nitrates readily convert to nitrites, which then react with the protein myoglobin to form nitric oxide myoglobin. During cooking, this is converted to nitrosohemochrome, a stable, pink pigment. They also impart a pink, fresh hue to cured meat. This chemical stabilises the red colour of the meat and gives an appearance of fresh meat. That is why nitrites are a favourite preservative of meat processors even though its excess use is restricted in many countries.

Precaution

Nitrite salts can react with certain amines in food to produce nitrosamines, which are known to cause cancer. Addition of Sodium ascorbate or sodium erythorbate inhibits nitrosamine formation and reduces the problem of nitrosamines.

The use of nitrite and nitrate has decreased greatly because of refrigeration and restrictions on the amounts used. Even though nitrite and nitrate cause only a small risk, it is always better to have fresh meat and meat 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. Name four important organic acids used as chemical preservatives?

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   …….

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2. What precautions should be observed before using SO_2 as preservative?

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3. How does nitrates impart red colour to meat?

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12.5 GENERAL RULES FOR CHEMICAL PRESERVATION

- Chemical food preservatives have to be used only at a dosage level which is needed for a normal preservation and not more.
- "Reconditioning" of chemical preserved food, e.g. a new addition of preservative in order to stop a microbiological deterioration already occurred is not recommended.
- The use of chemical preservatives MUST be strictly limited to those substances which are recognised as being without harmful effects on human beings' health and are accepted by national and international standards and legislation.

Factors which determine/ influence the action of chemical food preservatives

a) Factors related to micro-organisms:

As a general rule it is possible to take the following facts as a basis:

- Sulphur dioxide and its derivatives can be considered as an "universal" preservative as; they have an antiseptic action on bacteria as well as on yeasts and molds;
- Benzoic acid and its derivatives have a preservative action which is stronger against bacteria than on yeasts and molds;
- Sorbic acid acts on molds and certain yeast species; in higher dosage levels it acts also on bacteria, except lactic and acetic ones;
- The initial number of microorganisms in the treated product determines the efficiency of the chemical preservative.
- The efficiency is less if the product has been contaminated because of preliminary careless hygienic treatment or an incipient alteration. Therefore, with a low initial number of microorganisms in the product, the preservative dosage level could be reduced.

b) Factors related to the product:

- Chemical composition of the product.
- *pH value of the product:* the efficiency of the majority of chemical preservatives is higher at lower pH values, i.e. when the medium is more acidic.
- *Physical presentation and size which the product is sliced to:* the chemical preservative's dispersion in food has an impact on its absorption and diffusion through cell membranes on microorganisms and this determines the preservation effect. Therefore, the smaller the slicing of the product, the higher the preservative action. Preservative dispersion is slowed down by viscous foods (concentrated fruit juices, etc.)

c) Miscellaneous factors

- *Temperature:* chemical preservative dosage level will be established as a function of product temperature and characteristics of the micro-flora;
- *Time:* at preservative dosage levels in employed in industrial practice, the time period needed in order to obtain a "chemical sterilisation" is a few weeks for benzoic acid and shorter for sulphurous acid.

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. What is general rule about the dosage level of chemical preservative?

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2. Name one universal preservative?

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3. How does particle size of product affect the efficiency of preservative?

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12.6 LET US SUM UP

In this unit you have learnt about the chemical preservatives. Herein we have discussed the characteristics of approved chemical preservatives. Various types of preservatives grouped according to their mode of action have been discussed. More stress has been laid on antimicrobial preservatives owing to their importance. Factor influencing the effectiveness of various preservatives have also been discussed. We hope that after reading this unit you will become

more conscious about reading the label of processed product before consuming it.

12.7 KEY WORDS

Food Spoilage : Undesirable change in flavours, odours appearances or texture of food.

Preservation : Safeguarding

Preservative : Additive

Anti microbial : Which act against microorganisms.

Anti oxidant : Which removes the oxygen.

GRAS : Generally recognized as safe.

12.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following points:

   Chemical food preservatives are substances which, under certain conditions, either delay the growth of microorganisms without necessarily destroying them. These are added in very low quantities and which do not alter the organoleptic and physico-chemical properties of the foods at all or only very little.

2. Your answer should include the following points:

   i) It should have GRAS (Generally recognized as safe) status.
   ii) It should be properly declared on the label of the food in which used.
   iii) It should be used only at approved dosage level.

3. Your answer should include the following points:

   In its petition for approval, the manufacturer must demonstrate that the preservative is safe for consumers, considering:

   - the probable amount of the preservative that will be consumed with the food product, or the amount of any substance formed in or on the food resulting from use of the preservative
   - the cumulative effect of the preservative in the diet
   - the potential toxicity (including cancer-causing) of the preservative when ingested by humans or animals.

Check Your Progress Exercise 2

1. Your answer should include the following points:

   The two important antioxidant preservatives are:

   - butylated hydroxytoluene
   - butylated hydroxyanisole

2. Your answer should include the following points:

   Ascorbic acid (vitamin C) inhibit phenolase by making the pH uncomfortably low for the enzyme.

3. Your answer should include the following points:

   - nisin
   - natamycin

4. Your answer should include the following point:

   There is no limit for traditional food preservatives like salt and sugar.

Check Your Progress Exercise 3

1. Your answer should include the following points:

   The four important organic acids used as chemical preservatives are:

   - Benzoic Acid
   - Sorbic acid
   - Propionic acid
   - Acetic acid

2. Your answer should include the following points:

   1. Sulphites should not be used in foods that are important sources of thiamin (vitamin B1), such as enriched flour, because sulphites destroy the nutrient.
   2. It should not be consumed by asthmatic patients because it may cause severe allergic reactions.

3. Your answer should include the following points:

   Nitrates readily convert to nitrites, which then react with the protein myoglobin to form nitric oxide myoglobin. During cooking, this is converted to nitrosohemochrome, a stable, pink pigment. That is how they impart a pink, fresh colour to cured meat.

Check Your Progress Exercise 4

1. Your answer should include the following points:

   Chemical food preservatives have to be used only at a dosage level which is needed for a normal preservation and not more.

2. Your answer should include the following point:

   Potassium meta bisulphate

3. Your answer should include the following points:

   - The smaller is the slicing of the product, the higher is the preservative action.
   - Preservative dispersion is slowed down by viscous foods

12.9 SOME USEFUL BOOKS

1. Adams, M.R. and Moss, M.O. (2000) Food Microbiology. Royal Society of Chemistry, Cambridge, U.K.
2. Branen, L.A. and Davidson, P.M. (1983) Antimicrobials in Food. Marcel Dekker, New York.
3. Igoe, R.S and Hui, Y.H. (1996) Dictionary of Food Ingredients – 3rd ed. Chapman & Hall, New York.
4. Jay, J.M. (2000) Modern Food Microbiology, Van Nostrand Company, New York.
5. Lewis, R.J. (1989) Food Additives Handbook. Van Nostrand Reinhold, New York.

UNIT 13 MICROBIAL

Structure

13.0 OBJECTIVES

After studying this unit, you should be able to explain:

- fresh fruits and vegetables;
- canned and processed food products; and
- drinking water.

13.1 INTRODUCTION

In this unit we will make you aware about the types of micro flora present on the surface of fresh fruits and vegetables, the sources of these microorganisms and the characteristics of pathogenic microorganisms. We will also brief you about quality standards for drinking water and the human pathogen present in contaminated water. Canning is an established way of food preservation. You will learn about the history of canning, spoilage of canned products with special reference to *Clostridium botulinum*. After that we will brief you about the microbial limits for processed foods.

13.2 MICROBIOLOGICAL PROFILE OF HARVESTED FRUITS AND VEGETABLES

The consumption of fresh fruits and vegetables is increasing as consumers strive to eat healthy diets. Global trade in fruits and vegetables and changing horticultural practices have enabled this year-round abundance to be possible, as well as adding new varieties of fresh produce to the market. During the last few decades pre-prepared minimally processed fruits and vegetables have become popular among the consumers. These products include pre-washed pre-cut salads items, grated vegetables, prepared fruit salads, or fruit combinations. Most of these products are generally eaten raw without further processing. Some products are packed in modified atmospheres to provide extension of shelf life both in relation to the potential acceptable quality and safety of the product.

Since minimum processing is required for fresh and fresh-cut fruits and vegetables, which omits any effective microbial elimination step, results in food products that naturally would carry microorganisms, some of which may be potentially hazardous to human health.

13.2.1 Sources of Microorganisms on Fresh Fruits and Vegetables

Fruits and vegetables can become contaminated whilst growing in fields, or during harvest, handling, processing, distribution and use. However, there are certain factors, which contribute to the microbiological contamination of these products with pathogens. Table 13.1 lists the sources of pathogenic microorganisms on fresh produce and conditions that influence their survival and growth.

Contamination can arise as a consequence of treating soil with organic fertilizers such as manure and sewage sludge and from irrigation water. Manure, bio-solids and irrigation water should be of a quality that does not introduce pathogens to the treated commodity. The potential of organic farming to contaminate fruits and vegetables with pathogens has to be investigated. Harvesting at the appropriate time and storing the harvested products under controlled conditions will help to retard growth of post-harvest spoilage and pathogenic microorganisms. Humid and warm storage conditions encourage the growth of microbial contaminants. The use of additional post-harvest procedures could reduce the contamination level of fruits and vegetables. Washing with water of potable quality can reduce the microbial load. Although a wide range of different agents are available for disinfecting/ sanitizing fresh produce their efficacy is variable and none is able to ensure elimination of pathogens. Fruits and vegetables carry a natural non-pathogenic epiphytic micro flora. During growth, harvest, transportation and further processing and handling the produce can, however, be contaminated with pathogens from human or animal sources. The microbial composition of the different forms of organic fertilizer will vary depending on its origin and further treatment. The quality of the water used for irrigation and as a carrier for plant protection products, fertilizers and frost protection products has to be related to the potential risk it can cause at a later stage. Technologies for irrigation are important for the control of spreading microbiological hazards. The use of drip irrigation instead of flooding or spray irrigation should reduce waterborne contamination and aerosols. However, heavy rains and wind may provide other opportunities for the transfer of microorganisms from soil to plant surfaces.

Table 13.1: Sources of pathogenic microorganisms on fresh produce and conditions that influence their survival and growth

Pre-harvest
• Soil • Irrigation water • Green or inadequately composted manure • Air (dust) • Wild and domestic animals • Human handling • Water for other uses (for example, pesticides, foliar treatments, growth hormones)
Post-harvest
• Human handling (workers, consumers) • Harvesting equipment • Transport containers (field to packing shed) • Air (dust) • Wash and rinse water • Sorting, packing, cutting and further-processing equipment • Ice • Transport vehicles • Improper storage (temperature, physical environment) • Improper packaging (includes new packaging technologies) • Cross contamination (other foods in storage, preparation and display areas) • Improper handling after wholesale or retail purchase • Cooling water (for example, hydro cooling)

13.2.2 Factors affecting Type and Number of Microorganism on Fresh Fruits and Vegetables

Fruits and vegetables normally carry a non-pathogenic epiphytic micro flora. The majority of bacteria found on the surface of plants are usually Gram-negative and belong either to the *Pseudomonas* group or to the *Enterobacteriaceae*. Many of these organisms are normally non-pathogenic for humans. The numbers of bacteria present will vary depending on seasonal and climatic variation and may range from 10^4 to 10^8 per gram. The inner tissues of fruits and vegetables are usually regarded as sterile. However, bacteria can be present in low numbers as a result of the uptake of water through certain irrigation or washing procedures. If these waters are contaminated with human pathogens these may also be introduced. The survival or growth of contaminating microorganisms is affected by intrinsic, extrinsic and processing factors. Factors of importance are nutrient composition, pH, presence of scales and fibers, redox potential, temperature and gaseous atmosphere. Mechanical shredding, cutting and slicing of the produce open the plant surfaces to microbial attack. About two thirds of the spoilage of fruits and vegetables is caused by molds. Members of the genera *Penicillium*, *Aspergillus*, *Sclerotinia*, *Botrytis* and *Rhizopus* are commonly

involved in this process. The spoilage is usually associated with cellulolytic or pectinolytic activity, which causes softening of tissues, and weakening of plant structures. These structures are important barriers to prevent growth in the products by contaminating microbes.

13.2.3 Human Pathogens Associated with Fresh Fruits and Vegetables

However, risk profile surveys on the microbiological contamination of fruits and vegetables eaten raw demonstrates, potential for a wide range of these products to become contaminated with microorganisms, including human pathogens. The range of microorganisms associated with outbreaks linked to fresh produce encompasses bacteria, viruses and parasites. Most of the reported outbreaks have been associated with bacterial contamination, particularly members of the *Enterobacteriaceae*. Of these, *Salmonella* and *Escherichia coli* O157 are of particular concern. Outbreaks of illness caused by bacteria, viruses and parasites have been linked epidemiologically to the consumption of a wide range of vegetables and, to a lesser extent fruits. Surveillance of vegetables has indicated that these foods can be contaminated with various bacterial pathogens, including *Salmonella*, *Shigella*, *E. coli* O157:H7, *Listeria monocytogenes* and *Campylobacter*. Table 13.2 shows the characteristics of some microbial pathogens that have been linked to outbreaks of fresh fruits and vegetable associated illness.

Table 13.2: Characteristics of some microbial pathogens that have been linked to outbreaks of produce associated illness

Microorganism	Typical Incubation Period	Symptoms	Infectious Dose (Number of cells)	Source
BACTERIA				
Clostridium botulinum	12 to 36 h	Nausea, vomiting, fatigue, dizziness, dryness of mouth and throat, muscle paralysis, difficulty in swallowing, double or blurred vision, drooping eyelids, and breathing difficulties	Intoxication growth and toxin production in food	Soil, lakes, streams, decaying vegetation, reptiles
Escherichia coli O157:H7	2 to 5 d	Bloody diarrhoea, abdominal pain. Can lead to hemolytic uremic syndrome and kidney failure especially in children and the elderly	10 to 1000	Animal feces, especially cattle, deer and human; cross contaminati on from raw meat
Salmonella spp.	18 to 72 h	Abdominal pain, diarrhoea, chills, fever, nausea, vomiting	10 to 100,000	Animal and human feces; cross contaminati on from raw

				meat, poultry, or eggs
Shigella spp.	1 to 3 d	Abdominal pain, diarrhoea, fever, vomiting	~10	Human feces
Listeria monocytogenes	1 d to 5 or more wk	Febrile gastroenteritis in healthy adults; may lead to spontaneous abortion or stillbirth in pregnant women; severe septicemia and meningitis in neonates and immuno-compromised adults; mortality may be 20 to 40%	Unknown dependent upon health of individual	Soil, food processing environ-ments
PARASITES				
Cryptosporidium spp.	1 to 12 d	Profuse watery diarrhoea, abdominal pain, anorexia, vomiting	~30	Animal and human feces
Cyclospora spp.	1 to 11 d	Watery diarrhoea, nausea, anorexia, abdominal cramps (duration 7 to 40 d)	Unknown, probably low	Others? specific environment al sources unknown at this time
VIRUSES				
Hepatitis A	25 to 30 d	Fever, malaise, anorexia, nausea, abdominal pain, jaundice, dark urine	10 to 50	Human feces and urine
Norwalk/ Norwalk-like virus	12 to 48 h	Vomiting diarrhoea, malaise, fever, nausea, abdominal cramps	Unknown, probably low	Human feces, vomitus

However, multiplication of the pathogen is also essential for causing any damage. Some microorganisms cause illness only when ingested in high numbers (for example, *Clostridium perfringens*), while in other cases, the infectious dose is thought to be dependent upon the susceptibility of the individual (most infectious agents). Illness due to *Staphylococcus aureus*, *Bacillus cereus*, or *Clostridium botulinum* is a result of the production of toxins in the food, and it is the toxins that are responsible (sometimes in the absence of viable cells) for symptoms of the disease. These toxins are only produced by multiplying cells. This requires favourable growth conditions.

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 sources of microbial contamination on fresh fruits and vegetables?

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2. What are the factors that affect the survival and growth of microorganisms on fruits?

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3. Name few fungi responsible for spoilage of fresh fruits and vegetables?

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4. Name two bacteria associated with outbreaks linked to fresh fruits and vegetables?

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13.3 STANDARDS OF WATER FOR HUMAN CONSUMPTION

Clean water is one of the most important needs of our bodies. It is a sad fact that something as essential to life as clean drinking water can no longer be granted to us. According to research articles and news, most tap and well water now are not safe for drinking due to heavy industrial and environmental

pollution. We have reached to a point that, all sources of our drinking water, including municipal water systems, wells, lakes, rivers, and even glaciers, contain some level of contamination.

13.3.1 Sources of Contaminants in Drinking Water

Several contaminants occur in nature that may present a health risk if they are found in drinking water. The various pollutant /contaminants are bacteria, viruses, uranium, radium, nitrate, arsenic, chromium and fluoride. Other sources of contamination are a result of human activity such as manufacturing or agriculture, or individual misuse. The following activities may cause harmful microorganisms and chemicals to enter the well water owner's water supply.

- Leakage from waste disposal, treatment, or storage sites.
- Discharges from factories, industrial sites, or sewage treatment facilities.
- Leaching from aerial or land application of pesticides and fertilizers on yards or fields.
- Accidental chemical spills.
- Leakage from underground storage tanks.

13.3.2 Contamination due to Harmful Microorganisms

The most common and widespread health risk associated with drinking water is microbial contamination, either directly or indirectly, by human or animal excreta and micro-organisms contained in faeces.

The pathogenic agents involved include bacteria, viruses and protozoa, which may cause diseases that vary in severity from mild gastroenteritis to severe and sometimes fatal diarroea, dysentry, hepatitis or typhoid fever. Most of them are widely distributed through out the world. Fecal contamination of drinking water is only one of the several faeco-oral mechanisms by which they can be transmitted from one person to another, or in some cases, from animal to people. The human pathogens potentially transmitted in drinking water are Bacteria viz. Escherichia coli,Salmonella, Shigella, Vibrio cholera, Yersinia enterocolitica, Campylobacter jejuni; viruses viz, Adenoviruses, Enterovirus, Hepatitis A, Hepatitis E, Norwalk virus, Rotavirus, and small round viruses; The parasites, Giardia, Cryptosporidium, Entamoeba histolytica and Drancunculus. The removal of these agents from drinking water should be given top priority.

Apart from the above said **pathogens of high health significance**, there are some more organisms that are present in environment and **not normally regarded as pathogen, may caue disease opportunistically**. When such organisms are present in water they cause infection predominantly among people whose local or general defence mechanisms are impaired. Those most likely to be at risk include the very old, the very young and patients in the hospitals,e.g. those with burns or immunosuppressive therapy, and those suffering from acquired immunodeficiency syndrome(AIDS). Water used by such patients for drinking or bathing, if it contains excessive number of these agents, may produce a variety of infections involving the skin and mucous membrane of the eye, ear, nose and throat. *Pseudomonas, Flavobacterium, Acinetobacter, Klebsiella* and *Serratia* are examples of such opportunistic pathogens. *Legionella* infects the lung. These organisms while clearly of medical importance, acquire public health significance only under certain conditions. There removal from drinking water may therefore be given moderate priority.

13.3.3 Drinking Water Standards

Microorganisms, including pathogenic organisms, may enter water supplies at every stage of the collection and distribution cycle. Emphasis should be placed on the need for an active watershed protection program, including an emergency plan for responding to major pollution events such as spills or contamination. Major quality requirements for drinking water are listed in Table 13.3.

Table 13.3: Drinking water standards

Micro-organism	Requirement
Cryptosporidium	System must remove 99% of *Cryptosporidium*
Giardia lamblia	99.9% killed
Heterotrophic Plate count (HPC)	Not more than 500 colonies per ml.
Total Coliform	Must not be detectatable in any 100 ml sample. In case of large supplies where sufficient samples are examined must not be present in 95% of the samples taken through out any 12 months period.
Fecal Coliform or *E.coli*	No fecal coliform is allowed.
Turbidity	At no time can turbidity go above 5 NTU (Nephlometric turbidity unit)
Viruses	99.99% killed / inactivated

Let us know in brief about the above contaminants.

Coliform bacteria

These are common in the environment and are generally not harmful. However, the presence of these bacteria in drinking water is usually a result of a problem with the treatment system or the pipes which distribute water, and indicates that the water may be contaminated with germs that can cause disease. **Fecal Coliform and *E.Coli*** are bacteria whose presence indicates that the water may be contaminated with human or animal wastes. Microbes in these wastes can cause short-term effects, such as diarrhoea, cramps, nausea, headaches, or other symptoms.

Turbidity has no health effects. However, turbidity can interfere with disinfection and provide a medium for microbial growth. Turbidity may indicate the presence of disease causing organisms. These organisms include bacteria, viruses, and parasites that can cause symptoms such as nausea, cramps, diarrhoea, and associated headaches.

Cryptosporidium is a parasite that enters lakes and rivers through sewage and animal waste. It causes cryptosporidiosis, a mild gastrointestinal disease. However, the disease can be severe or fatal for people with severely weakened immune systems.

Giardia lamblia is a parasite that enters lakes and rivers through sewage and animal waste. It causes gastrointestinal illness (e.g. diarrhoea, vomiting, cramps).

Hence, it is important that our drinking water does not contain any concentration of microorganisms, parasites or any other substance which constitutes a potential human health risk and it meets the minimum requirements (microbiological and chemical parameters and those relating to radioactivity) laid down by the directives**.**

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. Name some common diseases caused due to contaminated drinking water?

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2. Name various pollutant/Contaminants of water?

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3. List some important human pathogen transmitted by drinking water?

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4. Name some opportunist pathogens in drinking water?

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13.4 MICROBIOLOGY OF CANNED FOODS

Foods are processed for convenience and safety. Food processing involves procedures such as: drying, canning, freezing, and pasteurization. For example, drying is a process by which water is removed from the product, causing the product to become dehydrated. Since microorganisms need water to grow, without moisture, they can't flourish. Canning is a process where foods are put into a container and given a high heat treatment to make the product sterile. The process of canning, be it vegetables, meat, or seafood, makes food safe because all dangerous microorganisms are destroyed. The canning process was developed to preserve food safely and for long periods of time.

13.4.1 History of Canning

The canning process dates back to the late 18th century in France when the Emperor Napoleon Bonaparte, concerned about keeping his armies fed, offered a cash prize to whoever could develop a reliable method of food preservation. Nicholas Appert conceived the idea of preserving food in bottles, like wine. After 15 years of experimentation, he realized if food is sufficiently heated and sealed in an airtight container, it will not spoil. An Englishman, Peter Durand, took the process one step farther and developed a method of sealing food into unbreakable tin containers, which was perfected by Bryan Dorkin and John Hall, who set up the first commercial canning factory in England in 1813. As more and more of the world was explored, and as provisioning armies took on greater importance, the demand for canned foods grew. Thomas Kensett, who emigrated to the United States, established the first U.S. canning facility for oysters, meats, fruits and vegetables in New York in 1812. More than 50 years later, Louis Pasteur provided the explanation for canning's effectiveness when he was able to demonstrate that the growth of microorganisms is the cause of food spoilage.

13.4.2 Basic Principal of Canning

The basic principles of canning have not changed dramatically since Nicholas Appert and Peter Durand developed the process. Heat sufficient to destroy microorganisms is applied to foods packed into sealed or "airtight" containers. The canned foods are then heated under steam pressure at temperatures of 116-121°C. The amount of time needed for processing is different for each food, depending on the food's acidity, density and ability to transfer heat. For example, tomatoes require less time than green beans, while corn and pumpkin require far more time. Processing conditions are chosen to be the minimum needed to ensure that foods are commercially sterile, but retain the greatest flavour and nutrition.

13.4.3 Spoilage of Canned Products

Heated canned foods may undergo spoilage either due to chemical or biological reasons. The most common spoilage of canned foods is the hydrogen swells produced as a result of action of food acid with the metal can. Such spoilage occurs mostly due to imperfect tinning and lacquering of interior of the can used for canning acidic foods. Biological spoilage of canned foods by the microorganism may result either from the survival of the organisms after the heat treatment or leakage of the container permitting entrance of the microorganisms, Surviving organisms may be vegetative cells or spore formers depending upon the heat treatment. Acid foods are processed at a temperature around $100^{O}C$ which result in killing of all vegetative cells of bacteria yeast and molds.

13.4.4 *Clostridium botulinum* a Major Threat in Canned Products

Growth of the bacterium *Clostridium botulinum* in canned food may cause **botulism –** a deadly form of food poisoning. These bacteria exist either as spores or as vegetative cells. Botulism is an **intoxication** that is caused by the ingestion of a virulent nerve toxin produced by the growth of the gram positive, obligate anaerobe, spore-former ***Clostridium botulinum***. This bacterium appears to be a normal inhabitant of the soil, hence is ready contamination of most foods. The spores can survive harmlessly in soil and water for many years. When ideal conditions exist for growth, the spores produce vegetative cells which multiply rapidly and may produce a deadly toxin within 3 to 4 days of growth in an environment consisting of:

- a moist, low-acid food
- a temperature between 4°C and 49°C
- less than 2 percent oxygen

It is able to grow in **absence of oxygen** in a wide variety of foods and in so doing produces a **protein neural toxin**, two to three grams (an amount equivalent to the quantity of salt in the average salt shaker on your table) of which would be sufficient to kill human being. However, the organism will not grow in the presence of oxygen or nitrate salts and it does not produce the toxin at a pH below 4.7. Only one strain, which is found associated with marine organisms, is able to produce the toxin at refrigerator temperature. The toxin is destroyed by boiling it at 100^{o}C for 10 to 15 min. However, the spore requires a temperature of 121^{o}C for 15 min to kill it. The toxin acts by binding to nerve junctions and destroying the nerve. The symptoms, which occur usually within 12 to 36 hours, but which can take up to 8 days to appear, classically consist of double vision, dizziness, inability to speak, breathe or swallow. Death often occurs due to the inability to breath. The only treatment is the injection of *antitoxin* to the several varieties of the toxin. This treatment is only effective against free toxin, as once the toxin has bound to the nerves the damage is irreversible. **The entire canning process is built around ensuring that all spores of this bacterium contaminating any canned food are destroyed in the sterilization process.** Industry has a sterling record in that deaths from commercial-botulism are very rare. This is influenced by the fact that once a product is known to contain botulism toxin none of that product is ever again purchased by a customer. **The majority of botulism poisonings occur in HOME-CANNED FOODS** prepared by grandma or your favourite aunt. A rule of thumb is "READ THE CANNING DIRECTIONS" and if you think a food might contain the botulism toxin never tastes even the smallest drop of it!

Some interesting additional information about this disease is:

- Never feed **raw honey** to a child under the age of two because the botulism spores can grow in the immature gut and produce the toxin. This can not occur in the adult due to our gut micro flora which is absent in infants.

- The botulism toxin is being used to treat certain neurological conditions where nerves that shouldn't fire do. In these cases tiny quantities of the botulism toxin is injected into the nerve, which the toxin kills and cures the condition.

- Ducks and chickens often die from botulism poisoning by eating rotting material in which the bacterium has grown. However, vultures, which as

you know, eat disgusting rotten, stinking carrion, are immune to the toxin through evolution.

Botulinum spores are on most fresh food surfaces because they grow only in the absence of air, they are harmless on fresh foods. Botulinum spores are very hard to destroy at boiling-water temperatures; the higher the canner temperature, the more easily they are destroyed. Therefore, all low-acid foods should be sterilized at temperatures of 115°C to 121°C, attainable with pressure canners operated at 10 to 15 PSIG. PSIG means pounds per square inch of pressure as measured by gauge. At temperatures of 115°C to 121°C, the time needed to destroy bacteria in low-acid canned food ranges from 20 to 100 minutes. The exact time depends on the kind of food being canned, the way it is packed into jars, and the size of jars. The time needed to safely process low-acid foods in boiling-water canner ranges from 7 to 11 hours; the time needed to process acid foods in boiling water varies from 5 to 85 minutes.

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 Canning?

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2. Name the person who for the first time conceived the idea of canning?

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3. Name the factor important in deciding canning time?

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4. Why is *Closteridium botulinum* a major threat in canned products?

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13.5 MICROBIOLOGICAL STANDARDS FOR PROCESSED FOODS

By now you know that microbiological hazards are one of the biggest threats to food safety. With better understanding in microbiology and food safety, safety limits have been set for a range of pathogenic microorganisms in foods. This subunit presents the recommended microbiological guidelines for some ready-to-eat food. According to the Codex Alimentarius Commission of the United Nations, an international food standard setting authority, the functions of

microbiological guidelines include formulation of design requirements, indication of required and expected microbiological status of the food commodities, and the verification of efficacy of hygienic practice. These guidelines stipulate the safety limits of nine major food borne pathogens such as *Salmonella* species, *Listeria monocytogenes*, *E coli O157* and *Vibrio cholerae*, as well as providing a classification of microbiological quality of ready-to-eat food for reflecting the hygienic status of the food concerned.

13.5.1 Purpose of Microbiological Standards

Microbiological Guidelines are criteria indicating the microbiological condition of the food concerned so as to reflect its safety and quality. These standard lists the maximum permissible levels of food borne microorganisms that pose a risk to human health in nominated foods, or classes of foods. They can be introduced to the food industry to observe voluntarily or stipulated in legislation for compliance.

13.5.2 Sampling

The statistical validity of a microbiological examination increases with the number of field samples analysed. For regulatory purposes, **a minimum of 5 sample units** from a lot is generally specified for examination. The size of the samples taken should also be adequate to enable appropriate microbiological analyses to be undertaken. A minimum sample size of 100g or ml is commonly required. **A lot** is defined as a quantity of food or food units produced and handled under uniform conditions. This may be restricted to a food item produced from a particular production line or piece of equipment within a certain time period (not exceeding 24 hours).

13.5.3 Microbiological Assessment

There are three major components under microbiological assessment of any food.

Aerobic colony count is a count of viable bacteria based on counting of colonies grown in nutrient agar plate. This is commonly employed to indicate the sanitary quality of foods. The incubation condition of ACC used in this guideline is **30° C for 48 hours**.

Indicator organism Counts refers to the selected surrogate markers. The main objective of using bacteria as indicators is to reflect the hygienic quality of food. *E. coli* is commonly used as surrogate indicator. Its presence in food generally indicates direct or indirect fecal contamination. Substantial number of *E. coli* in food suggests a general lack of cleanliness in handling and improper storage. **Specific pathogens Counts** refer to bacteria that may cause food poisoning. Mechanisms involved may be toxins produced in food or intestinal infection. Nine specific bacterial pathogens are included in this set of guidelines. The symptoms of food poisoning vary from nausea and vomiting (e.g. caused by *S. aureus*), through diarrhoea and dehydration (*Salmonella* spp. and *Campylobacter* spp.) to paralysis and death in the rare cases of botulism. The infectious doses vary from less than 10 to more than 10^6 organisms.

13.5.4 Categories of Food based on Microbial Quality

For assessment of hygienic quality, food items are grouped into five categories taking into account the raw ingredients used, and the nature and degree of processing before sale. The microbiological assessment of ready-to-eat food on the above three components will lead to the classification of the food quality into one of the following four classes:

a) **Class A:** the microbiological status of the food sample is **satisfactory.**

b) **Class B:** the microbiological status of the food sample is **less than satisfactory but still acceptable for consumption.**

c) **Class C:** the microbiological status of the food sample is **unsatisfactory**. This may indicate a sub-optimal hygienic conditions and microbiological safety levels. Licensees of food premises should be advised to investigate and find out the causes and to adopt measures to improve the hygienic conditions. Taking of follow-up samples to verify the improvement may be required.

d) **Class D:** the microbiological status of the food sample is unacceptable. The food sample contains unacceptable levels of specific pathogens that is **potentially hazardous to the consumer**. In addition to giving advice to the licensee of the food premises as stated in (c) above, warning letters as well as other enforcement actions should be considered. Microbiological limits in respect of the above components are summarized in the table 13.4.

Table 13.4: Guideline levels for determining the microbiological quality of ready-to-eat foods

Criterions		**Microbiological Quality (CFU per gram)**			
		Class A Satisfactory	**Class B Marginal**	**Class C Unsatisfactory**	**Class D Potentially Hazardous**
Aerobic colony count (ACC)[30^OC/48 hr]					
Food Category	**1**	$<10^3$	10^3-$<10^4$	$\geq 10^4$	N/A
	2	$<10^4$	10^4-$<10^5$	$\geq 10^5$	N/A

(Food items detailed in Table 5)	**3**	$<10^5$	10^5-$<10^6$	$\geq 10^6$	N/A
	4	$<10^6$	10^6-$<10^7$	$\geq 10^7$	N/A
	5	N/A	N/A	N/A	N/A
Indicator organisms (Apply to all food categories)					
E.coli (Total)		<20	20 -< 100	≥100	N/A
Pathogens (Apply to all food categories)					
Campylobacter spp		not detected in 25g	N/A	N/A	Present in 25 g
Escherichia coli 0157		not detected in 25g	N/A	N/A	Present in 25 g
Listeria monocytogenes		not detected in 25g	N/A	N/A	Present in 25 g
Salmonella spp		not detected in 25g	N/A	N/A	Present in 25 g
Vibrio cholerae		not detected in 25g	N/A	N/A	Present in 25 g
Clostridium perfringens		<20	20<100	$100<10^3$	$\geq 10^3$
Staphylococcus aureus		<20	20<100	$100<10^4$	$\geq 10^4$
Vibrio parahaemolyticus		<20	20<100	$100<10^4$	$\geq 10^4$
Bacillus cereus		$<10^3$	10^3-$<10^4$	10^4-$<10^5$	$\geq 10^5$

N/A Not applicable

The desired microbiological quality of the some food samples is summarized in Table 13.5.

Table 13.5: Food category table for aerobic colony count assessment

Food group	Food item	Category
Meat	Beefburgers and kebabs	1
	Dim sum	2
	Pate (meat, seafood or vegetable)	3
	Poultry (unsliced)	2
	Preserved meat	4
	Salami and fermented meat products	5
	Sausages	2
	Smoked meat	5
	Siu-mei & lo-mei	3
	Sliced meat (ham and tongue) (cold)	4
	Sliced meat (beef, haslet, pork, poultry, etc.) (dried)	3
	Steak and kidney / meat pies	2
	Tripe and other offal	4
Seafood	Crustaceans	3
	Pickled fish	1
	Other fish (cooked)	3
	Oysters (raw)	5
	Seafood meals	3
	Shellfish (cooked)	4
	Smoked fish	4
Dessert	Cakes, pastries, slices and desserts – with dairy cream	3
	Cakes, pastries, slices and desserts – without dairy cream	2
	Cheesecake	5
	Mousse / dessert	1
	Tarts, flans and pies	2
	Trifle	3
Savoury	Bean curd	5
	Cheese-based bakery products	2
	Fermented foods	5
	Flan / quiche	2
	Dips	4
	Mayonnaise / dressings	2
	Samosa	2
	Satay	3
	Spring rolls	3
Vegetable	Coleslaw / salads (with or without meat)	3
	Fruit and vegetables (dried)	3
	Fruit and vegetables (fresh)	5
	Rice	3

	Vegetables and vegetable meals (cooked)	2
Dairy	Cheese Yoghurt	5 5
Ready-to-eat meals	Pasta / pizza Meals (others)	2 2
Sandwiches and filled rolls	With salad Without salad	4 3
Sushi & sashimi	Fish fillet and fish roe sashimi / sushi Sashimi other than fish fillet and fish roe	3 4

Controlling microbes

Control of microbes in processed products primarily depend upon good manufacturing practices and one of the most effective way to ensure this is by application of HACCP. HACCP stands for Hazard Analysis of Critical Control Point. HACCP is a preventive system for assuring production of safe food. It is a process that identifies food safety hazards associated with a product and process and strictly manages and monitors the Critical Control Points (CCP's) designed to control the hazard as a way of ensuring the process is in control and that the safest product possible is being produced. It requires establishment of hazard, identification of critical control points, effective monitoring follow up and evaluation. For a food processor it is necessary to know the microbial quality of the raw material, the processing environment, and the packaging component. This also requires validation of all processing stages designed to destroy both the pathogens and the spoilage agents and the efficacy of preservative system.

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. What is codex Alimentarius?

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2. Why do we need microbial standards?

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3. How many sample units from a lot are generally specified for examination?

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4. What are the major components under microbiological assessment of any food?

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5. Define Aerobic colony count?

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6. What does the presence of indicator organism in food reflect?

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7. Name the specific pathogens to be observed for microbial assessment of any food?

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13.6 LET US SUM UP

In this unit you have learnt about the microbial quality limits for various foods (raw and processed). Herein we have discussed the sources of microorganisms on fresh fruits and vegetables and in drinking water. Various Food and water borne harmful microorganisms including indicator bacteria, viruses and pathogens have been discussed briefly. *Clostridium botulinum* is an important organism for canning point of view. Hence, it has been dealt in more detail. WE HOPE that after reading this unit you will become more conscious about the microbial quality of food that you are going to eat.

13.7 KEY WORDS

Spoilage microorganism : Microorganism which spoil the product by developing undesirable flavours, odours and changing food appearances or textures via microbial action.

Pathogenic : Microorganism which may infect plants, animals and man and make them sick.

Hazardous : Harmful

Epidemic : Out break of infectious disease

Gastroenteritis : Inflamatory change of lining of stomach caused by microorganism ingested with food and water.

Hepatitis : Virus Hepatitis A

Parasites : Life on, within or at the expense of other organisms.

Toxin : Poison

Intoxication : Toxin production

13.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1. Your answer should include the following point:

   - Sources of microorganisms on fresh fruit are air, orchard soil, irrigation water, harvesting device, storing and packaging containers, handling personnel etc.

2. Your answer should include the following points:

    - The survival or growth of contaminating microorganisms is affected by intrinsic, extrinsic and processing factors.
    - Factors of importance are nutrient composition, pH, presence of scales and fibers, redox potential, temperature and gaseous atmosphere.
    - Mechanical shredding, cutting and slicing of the produce open the plant surfaces to microbial attack.

3. Your answer should include the following point:

    - Members of the genera *Penicillium*, *Aspergillus*, *Sclerotinia*, *Botrytis* and *Rhizopus* are commonly involved in spoilage of fresh fruits and vegetables.

4. Your answer should include the following points:

    - Most of the reported outbreaks have been associated with bacterial contamination.
    - Members of the *Enterobacteriaceae*. Of these, *Salmonella* and *Escherichia coli* O157 are of particular concern.

Check Your Progress Exercise 2

1. Your answer should include the following points:

    - Gastroenteritis
    - Diarroea
    - Dysentry
    - Hepatitis
    - Typhoid fever

2. Your answer should include the following points:

    - Microbes
    - Radionuclide
    - Inorganics
    - Volatile organics
    - Disinfectants
    - Disinfection by products etc.

3. Your answer should include the following points:

    - The human pathogens potentially transmitted in drinking water are Bacteria viz. *Escherichia coli,Salmonella, Shigella, Vibrio cholera, Yersinia enterocolitica, Campylobacter jejuni.*
    - Viruses viz, Adenoviruses, Enterovirus, Hepatitis A, Hepatitis E, Norwalk virus, Rotavirus, and small round viruses
    - Parasites viz. *Giardia*, *Cryptosporidium*, *Entamoeba histolytica* and *Drancunculus*.

4. Your answer should include the following points:

    - *Pseudomonas*

- *Flavobacterium*
- *Acinetobacter*
- *Klebsiella*
- *Serratia*
- *Legionella*.

Check Your Progress Exercise 3

1. Your answer should include the following point:

   - Process where foods are put into a container and given a high heat treatment to make the product sterile.

2. Your answer should include the following point:

   - Nicholas Appert

3. Your answer should include the following points:

   - Food's acidity
   - Density
   - Ability to transfer heat.

4. Your answer should include the following point:

   - Growth of the bacterium *Clostridium botulinum* in canned food may cause **botulism –** a deadly form of food poisoning.

Check Your Progress Exercise 4

1. Your answer should include the following point:

   - Codex Alimentarius is an international food standard setting authority.

2. Your answer should include the following points:

   - Lists the maximum permissible levels of food borne micro-organisms that pose a risk to human health in nominated foods, or classes of foods.
   - Indicate the microbiological condition of the food concerned so as to reflect its safety and quality.

3. Your answer should include the following point:

   - Minimum of 5 sample units from a lot is generally specified for examination.

4. Your answer should include the following points:

   - Aerobic colony count
   - Indicator organism Counts
   - Specific pathogens Counts

5. Your answer should include the following points:

- Count of viable bacteria based on counting of colonies grown in nutrient agar plate.
- Commonly employed to indicate the sanitary quality of foods.

6. Your answer should include the following point:

   - The presence of indicator organisms in food reflect the hygienic quality of food.

7. Your answer should include the following points:

   - *Campylobacter* spp.
   - *Escherichia coli* 0157
   - *Listeria monocytogenes*
   - Salmonella spp
   - *Vibrio cholerae*
   - *Clostridium perfringens*
   - *Staphylococcus aureus*
   - *Vibrio parahaemolyticus*
   - *Bacillus cereus*

13.9 SOME USEFUL BOOKS

1. Adams, M.R. and Moss, M.O. (2000) Food Microbiology. Royal Society of Chemistry, Cambridge, U.K.

2. Jay, J.M. (2000) Modern Food Microbiology, Van Nostrand Company, New York.

EXPERIMENT 1 PREPARATION OF MEDIA

Structure

1.1 INTRODUCTION

Microorganisms require certain basic nutrients and physical factors for the sustenance of life as do all other living organisms. However, their particular requirements may vary greatly. Nutritional needs of microbial cells are supplied in the laboratory through a variety of media to detect their presence. Microbiological media for the evaluation of spoilage and detection of bacteria, yeasts and molds in foods are mentioned in this chapter.

Objectives

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

- learn that microorganisms are ubiquitous and in nature, they do not segregate themselves by species but exist a mixture of many other cell types; and
- learn to make different media which are suitable for a particular genera of microorganisms to produce their discrete colonies.

1.2 EXPERIMENT

1.2.1 Principle

Many special purpose media are needed to facilitate recognition, enumeration and isolation of different types of microorganisms. To meet these needs, the microbiologists has developed numerous media which on the basis of their function may be classified as follows:

A) Selective media

These media provide nutrients that enhance the growth and predominance of a particular type of bacterium and do not enhance (and may even inhibit) other types of organisms that may be present. For instance, a medium in which cellulose is the only carbon source, will specifically select for, or enrich the growth of cellulose-utilizing organisms when it is inoculated with a soil sample containing many kinds of bacteria.

B) Differential media

Certain reagents or supplements, when incorporated into culture media, may allow differentiation of various kinds of microorganisms. For example, if a mixture of bacteria is inoculated on to a blood containing

agar medium (blood agar) some of the bacteria may hemolyze (destroy) the red blood cells, others do not. Thus one can distinguish between hemolytic and non-hemolytic bacteria on the same media.

1.2.2 Requirements (Equipment/Machinery/Instrument/Chemicals/ Material)

- Autoclave (Portable)
- Balance
- Heating mantle/ water bath
- pH meter
- Laminar air flow
- Stirrer
- Pipettes
- Distilled water
- Media (nutrient agar, potato dextrose agar, violet red bile agar, plate count agar)
- Test tubes
- Beakers
- Cotton plugs
- pH paper
- Measuring cylinder

1.2.3 Procedure

The preparation of microbiological media usually involves the following steps:

1. Carefully weigh the proper amount of the dehydrated base medium or the correct proportion of constituent ingredients and dissolve in appropriate volume of distilled water and heat. Composition of the media is as given in Annexure-1.

2. Determine the pH of the medium, and adjust if necessary with dilute acid or alkali.

3. If a solid medium is desired, add agar (1.5-2%) and boil the medium to dissolve the agar.

4. Distribute the medium into tubes or flasks. The amount of medium distributed per container should be limited so that no point within the volume of the medium is more than 2.5 cm from the top surface of the container.

5. Autoclave at 121°C for 15 minutes. Some media (or specific ingredients) that are heat labile are sterilized by filtration.

1.2.4 Observations

Observe the pH using the pH meter or colour indicator solutions. At the next laboratory period observe for any contamination to ensure proper sterilization. Discard any contaminated flasks and wash them in disinfectant solution.

1.2.5 Results

After performing this experiment you will observe that suitable media for growth of bacteria is nutrient agar (pH 6.8-7.0) or plate count agar (pH 7.0), whereas for yeasts and moulds it is potato dextrose agar (pH 5.6) and malt agar (pH 5.4).

1.3 PRECAUTIONS

- Adjust the pH of the media accurately, to provide favourable condition of growth for the microorganisms. pH of the medium may change during sterilization and because of possible browning reactions, it is important not to exceed the recommended time and temperature.
- Prepare medium in such quantities that if stored, it will be used before loss of moisture through evaporation that becomes evident.
- To prevent contamination and excess evaporation of water from a medium in flask and tubes during storage, optionally fit aluminium foil or plastic with loose rubber bands before autoclaving in order to allow air to escape and to prevent the container from bursting.
- Avoid over loading autoclaves so that the rate of air exhaust and heating is not appreciably delayed. The autoclave should reach 121°C (15 psi) slowly but within 10 min. after starting the air exhaust operation.
- Flask or test tubes should be plugged with cotton or capped with paper.
- After sterilization gradually reduce the pressure within the autoclave (using no less than 15min) since liquids may be at a temperature above their boiling point at atmospheric pressure. If the pressure is lowered too rapidly, liquids may boil over and come out from the container.
- Used plates, pipettes, tubes etc. should be routinely decontaminated by autoclaving for 30 minute at 121°C.
- Media should be stored at 2-8°C in a dry, dust free area and should not be exposed to direct sunlight.

EXPERIMENT 2 MICROSCOPIC STAINING TECHNIQUES

Structure

2.1 INTRODUCTION

Visualization of microorganisms in the living state is most difficult, not only because they are minute but also because they are transparent and practically colourless when suspended in an aqueous medium. To study their properties and to differentiate microorganism into specific groups for diagnostic purposes, biological stains and staining procedures in conjunction with light microscopy have become major tools in microbiology. Chemically a stain may be defined as an organic compound containing a benzene ring, a chromophore (chemical group that imparts colour to benzene), and an auxochrome (chemical compound that helps in binding to cells).

Objectives

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

- learn the practical and theoretical basis of chemical staining;
- describe manipulative technique of smear preparation;
- explain procedures for simple staining and negative staining; and
- perform differential staining procedures such as the Gram's staining, acid fast staining and spore staining.

2.2 EXPERIMENT

2.2.1 Principle

Staining by various dyes provides contrast between microorganisms and their background, permitting differentiation among various morphological types and internal structure such as cell wall, vacuoles or nuclear bodies. It also enables the microbiologist to use higher magnifications.

Numerous staining techniques are available for visualization, differentiation and separation of bacteria in terms of morphological characteristics and cellular structures. A summary of commonly used procedures and their purpose is outlined in Figure 2.1.

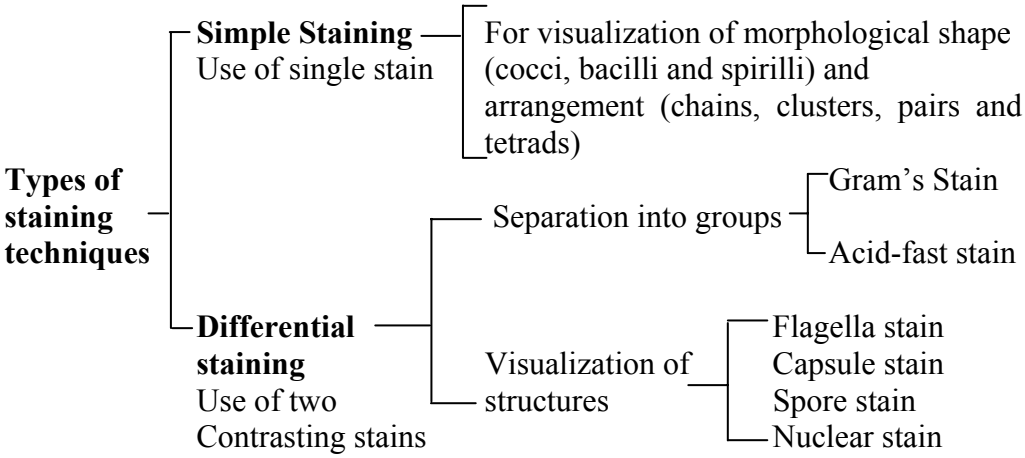


Figure 1: Staining techniques

2.2.2 Requirements (Equipment /Machinery/Instrument and Chemicals/ Material)

- Bunsen Burner
- Microscope
- Test tube shaker
- Inoculating needle
- Cover slips
- Glass slides
- Sterilized test tubes
- Wash bottles
- Microbial cultures
- Distilled water
- Stains
- Immersion oil
- Tissue paper

2.2.3 Procedure

Preparation and fixation of bacteria for staining

Prior to staining, you must "fix" the material to be observed that is make it stick to the glass slide upon which is to be stained. If a preparation is not fixed, the film of cells will wash off during the staining procedure. Purpose of fixation is also to kill the microorganism and coagulate the protoplasm of the cell so as to fix it on glass surface (Figure 2.2).

The fixing technique, although not difficult, requires adequate care in its preparation. Follow these basic rules meticulously:

1. ***Preparation of glass slides:*** Clean slides are essential for preparation of microbial smears. Grease or oil from fingers on slides must be removed by washing the slides with soap and water, followed by a water rinse. After cleaning dry the slides and place them on laboratory towels until ready for use.

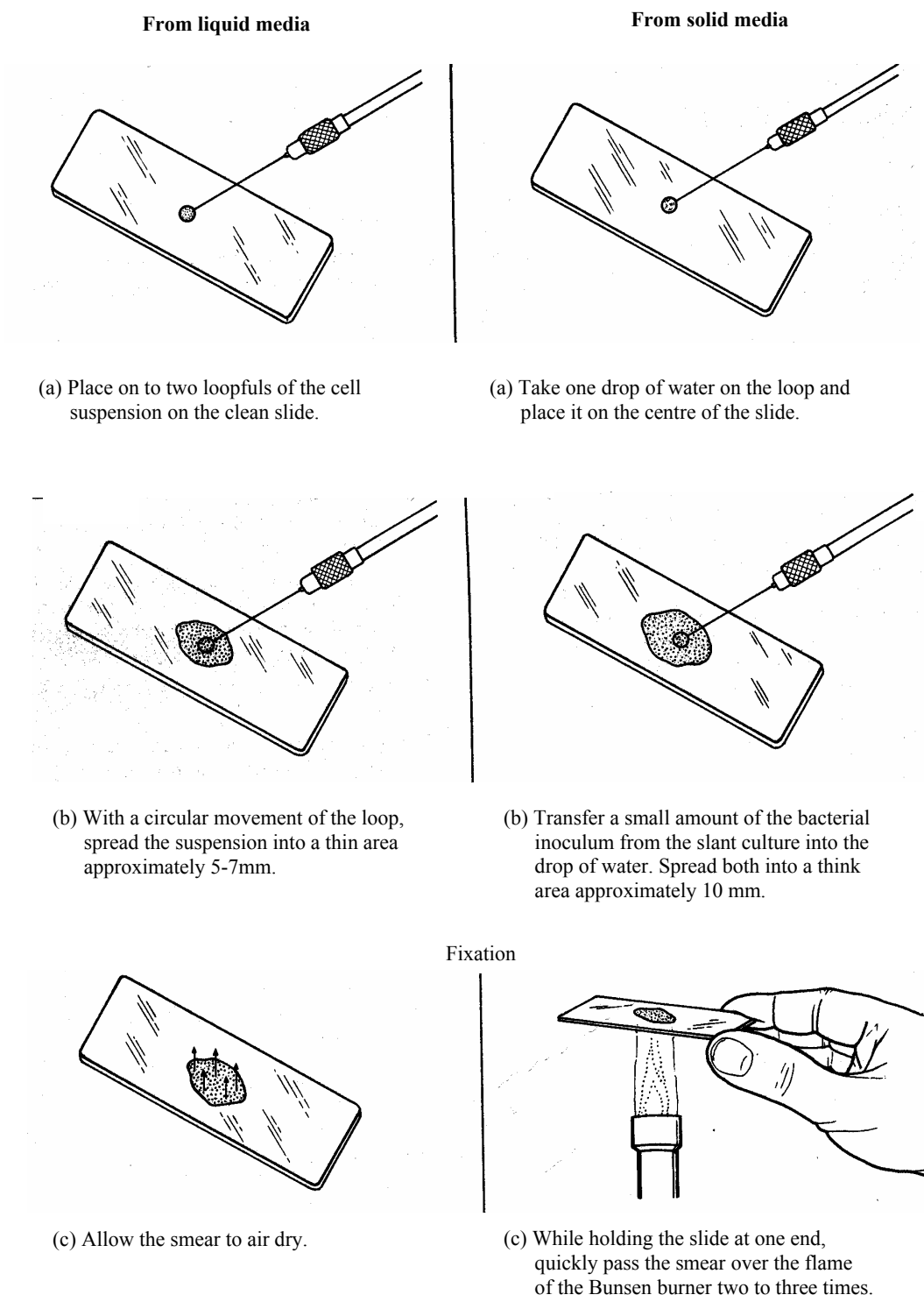


Figure 2.2: Bacterial smear preparation

2. ***Preparation of smear:*** Avoidance of thick, dense smears is absolutely essential. A good smear is one that, when dried, appears as a thin whitish layer or film. Those made from broth cultures or cultures from a solid medium require variations in technique.

    - *Broth cultures:* One or two loopful of suspended cells should be applied directly to the glass slide with a sterile inoculating loop and spread evenly over a small area.

    - *Cultures from a solid media:* Organisms cultured in a solid medium produce thick, dense surface growth and are not amenable to direct transfer to the glass slide. These cultures must be diluted by placing a

loopful of water on the slide in which the cells will then be emulsified. Suspension is accomplished by spreading the cells in a circular motion in the drop of water with the needle tip. At this point, the smear must be allowed to dry completely. **Do not blow or wave it in the air.**

3. ***Heat fixation:*** Unless fixed on the glass slide, the bacterial smear will wash away during the staining procedure. This is avoided by heat fixation, during which the bacterial proteins are coagulated and fixed to the glass surface. Heat fixation is performed by the rapid passage of the air-dried smear two or three times over the flame of the Bunsen burner.

Staining with basic dyes

Herein, the bacterial smear is stained with a single basic stain. The bacterial nucleic acid and certain cell wall components carry a negative charge that strongly attract and bind to the cationic (negatively charged) chromogen. The purpose of simple staining is to elucidate the morphology and arrangement of bacteria.

The most commonly used basic stains are methylene blue, crystal violet and carbol fuchsin. Note that exposure time for staining cells to these dyes differs for each of these stains; carbol fuchsin requires 15-30 seconds, crystal violet 20-60 seconds and methylene blue 1-2 minutes for fresh cultures. For old cultures more time is required for staining.

General staining

Procedure for staining with different dyes:

1. Prepare bacterial smear of the organisms. Note: All smears must be heat fixed prior to staining.
2. Flood the smear with any one of the stains, using the appropriate exposure time.
3. Wash the stained preparation with tap water to remove excess stain. During this step, hold the slide parallel to the stream of water; in this way you can reduce the loss of organisms from the preparation.
4. Dry the slide using blotting paper.
5. Examine the stained preparation under the oil-immersion objective of the microscope.

Observe closely for significant difference in cell size, shape and arrangements.

Negative or indirect staining

1. Place a small drop of nigrosin close to one end of a clean slide.
2. Using sterile technique, place a loopful of inoculum from the mixed culture in the drop of nigrosin and mix.
3. With the edge of the second slide held at above 30° angle and placed in front of the bacterial surface, push the mixture to from a thin smear.
4. Air dry. Do not heat fixed slide.
5. Examine the slide under oil-immersion objective of the microscope.

Differential staining

Gram's stain

1. Prepare smear of the bacterial culture. Air-dry and fix these preparations with heat.
2. Flood smear with crystal violet and let stained for 30 seconds.
3. Rinse with water.
4. Cover the film with Gram's Iodine instantly and let stained for 1 min.
5. Wash with tap water.
6. Decolorize with 95% alcohol. For a thin smear, 10-20 second is long enough.
   Caution: Do not over-decolorize. Add reagent drop by drop until crystal violet fails to wash from smear.
7. Rinse with water.
8. Counter stain with safranine for 20-30 seconds.
9. Rinse with water and blot dry.
10. Examine under the oil-immersion objective.

Table 1: Steps in the gram's stain

Step	Procedure	Results	
		Gram +	Gram -
Initial Stain	Crystal Stain for 30 seconds	Stains purple	Stains purple
Mordant	Iodine for 30 seconds	Remains purple	Remains purple
Decolourization	95% ethanol for 10-20 seconds	Remains purple	Becomes colourless
Counterstain	Safranine for 20-30 seconds	Remains purple	Stains pink

Acid fast stain

1. Prepare a smear of bacterial culture.
2. Allow to air dry and heat fix in usual manner.
3. Flood smear with carbol fuchsin and place on a warm hot plate, allowing the preparation to steam for 5 minutes. **Caution**: Do not allow stain to evaporate, replenish stain as needed. Also prevent stain from boiling by adjusting the hot plate to a proper temperature.
4. Wash with tap water. Heated slides must be cooled prior to washing.
5. Decolorize with acidic alcohol (95% ethyl alcohol containing 2.5% HNO_3) for 10-30 seconds, a carbol fuchsin fails to wash from smear.
6. Wash with water.

7. Counter stain with methylene blue for 2 min.
8. Wash smear with tap water and blot dry.
9. Examine under the oil immersion objective.

Structural stain

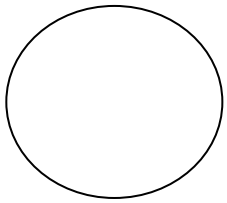
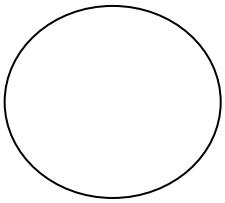
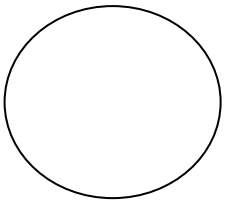
Endospore stain

1. Prepare smear, air dry and fix with heat.
2. Flood smear with malachite green and place on a warm hot plate, allowing the preparation to steam for 2-3 minute.
   Caution: Do not allow stain to evaporate; replenish stain as needed. Prevent the stain from boiling by adjusting the hot plate at a proper temperature.
3. Cool slide and wash with water.
4. Counter stain with safranine for 30 min.
5. Wash with water and blot dry.
6. Examine under oil immersion objective.

2.2.4 Observations

In the space provided:

1. Draw a representative field for each organism
2. Describe the morphology of the organism with reference to their shape (bacilli, cocci, spirilli) and arrangements (chains, clusters, pairs)

Stain	Methylene blue	Gram Stain	Carbol Fuschin
Drawing of a representative field			
Cell morphology			
Arrangement			
Cell colour			

2.2.5 Results

Staining the microorganisms makes them contrast in colour with their surroundings so that they are more readily visible. Certain stains can also be used to identify certain structures of the cell which would otherwise be unseen.

2.3 PRECAUTIONS

- Clean, dry glass slide must be taken to prepare a smear.
- Thick dense smears should be avoided.
- The smear should be properly heat fixed on the slide to avoid its washing off during staining procedure.
- Do not heat fix in case of negative staining.
- Do not over decolorize in case of Gram's staining.
- Do not allow stain to evaporate while acid staining technique. Replenish stain as needed.

EXPERIMENT 3 CULTURING AND IDENTIFICATION OF MICROORGANISMS

Structure

3.1 INTRODUCTION

Microorganisms are ubiquitous. They are found in soil, air, water, food, sewage and body surfaces. In short, every area of our environment is replete with them. When grown on a variety of media, microorganisms will exhibit differences in the microscopic appearance of their growth. These differences, called cultural characteristics, are used as basis for separating microorganisms into taxonomic groups. The cultural characteristics for all non-microorganisms are contained in Bergy's Manual of Systemic Bacteriology with their morphological characteristics.

Objectives

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

- differentiate microorganisms into bacteria, yeasts and moulds; and
- know the different forms / shapes of microorganisms.

3.2 EXPERIMENT

3.2.1 Principle

The microorganisms can be divided into bacteria, yeasts and moulds on basis of the difference in their morphological, cultural and physiological characteristics.

Bacteria

Among the major characteristics of bacterial cells are their size, shape, structure and arrangement. These characteristics constitute the morphology of the cell. Bacteria are very small, most being approximately 0.5 to 1.0 micrometers in diameter. They are unicellular, have cell wall and cytoplasm but the nucleous is not well developed. The shape of a bacterium is governed by its rigid cell wall. Typical bacterial cells are spherical (cocci), straight rods (bacilli) or rods that are helically covered (spirilla).

Different patterns for arrangement for identification purposes are monococci, diplococci, streptococci, tetrads, staphylococci and sarcinae (Figure 3.1). Cocci generally reproduce by binary fission. Rod shaped bacteria may be sporulating type like *Bacillus* species and *Clostridium* species which produce endospores or they are non-sporulating like *Lactobacillus* species (Figure 3.2). Bacteria may be both motile (having flagella) or non-motile (no flagella).

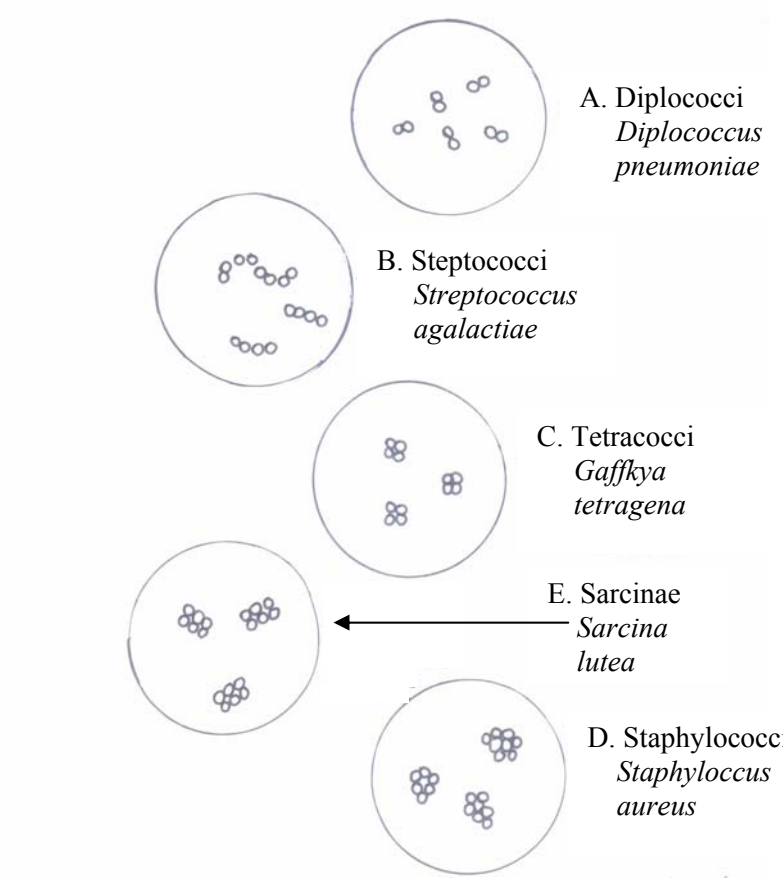


Figure 3.1: Characteristic arrangements of cocci

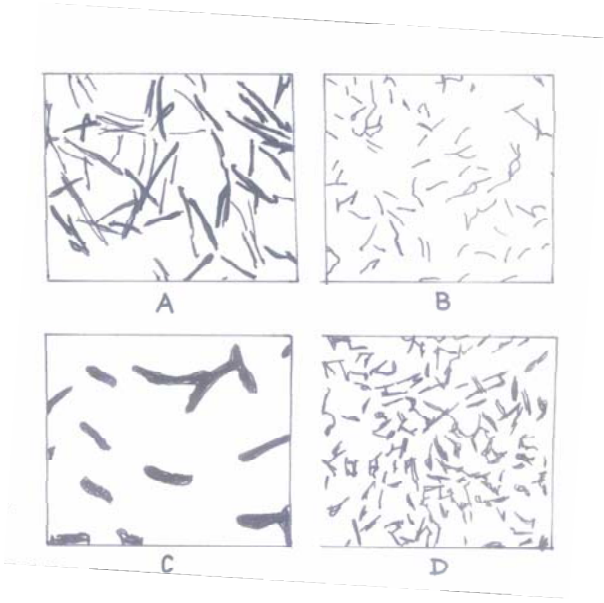


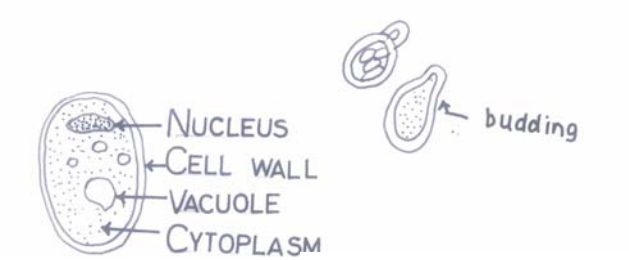
Figure 3.2: Types of rod-shaped bacteria. A) *Clostridium sporogenes*; B) *Pseudomonas sp.*; C) *Bacillus megaterium*; and D) *Salmonella typhi*

Fungi

Fungi is a group of eukaryotic organisms. They comprise of yeasts and moulds. Whereas moulds are filamentous and multicellular, yeasts are unicellular.

Yeasts

In general yeast cells are larger than most bacteria. Yeasts vary considerably in size ranging from 1-5 micrometer in width and from 5-30 micrometer in length. They are commonly egg-shaped, but some are elongated and some spherical. Yeasts lack flagella and other means of locomotion (Figure 3.3).



Saccharomyces sp. (a common bread yeast)

Figure 3.3: Yeast cell

Moulds

The thallus of moulds consist essentially of two parts: the mycelium and the spores. The mycelium is a complex of several filaments called hyphae. Filaments are made up of cells arranged end to end, branched and intertwined. Cells are like cells of higher plants in that they have visible nuclei, cell wall of varying thickness and cytoplasm. Mycelia in some fungi are divided into individual cells separated by cross walls and each cell containing a nucleus (Figure 3.4 and 3.5).

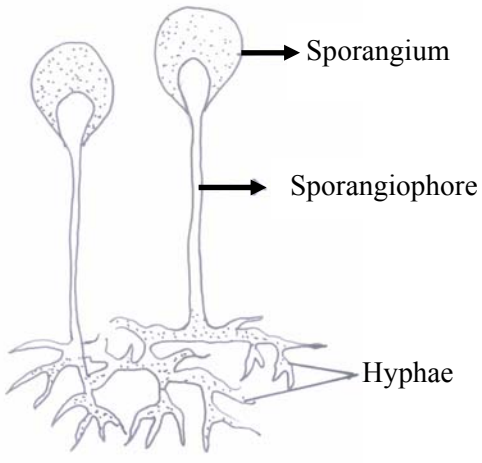


Figure 3.4: *Mucor* sp.

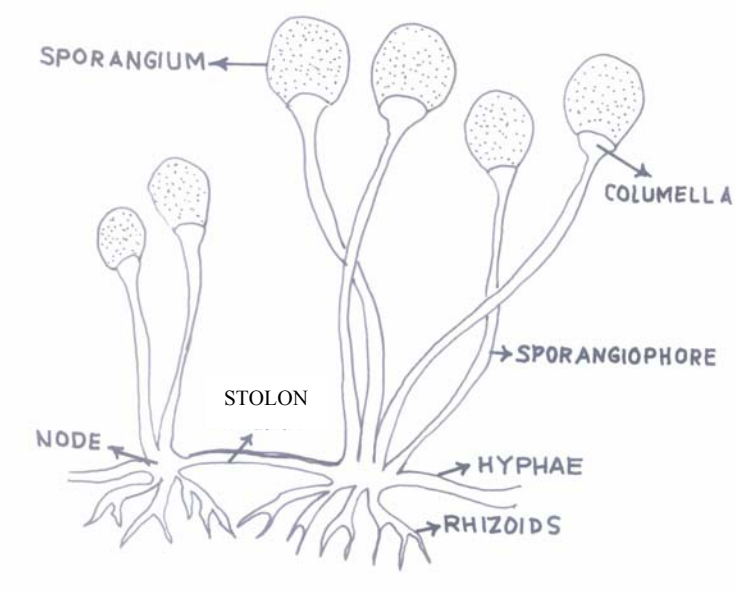


Figure 3.5: *Rhizopus* sp.

3.2.2 Requirements (Equipment/ Machinery/ Instrument and Chemicals/ Material)

- Compound microscope
- Bunsen burner
- Immersion oil
- Glass slides
- Inoculating needle
- Cover slips
- Tissue paper
- Microbial culture
- Distilled water

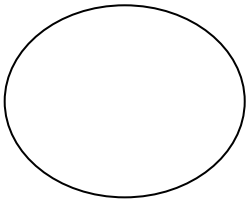
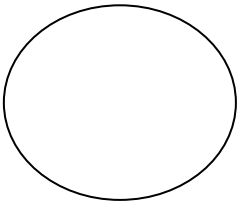
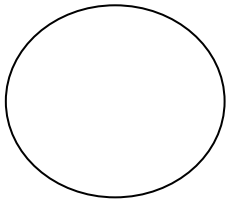
3.2.3 Procedure

1. Prepare the required media (broth or agar) for culturing the microorganisms
2. Place a small amount of media into test tubes, plug and sterilize them in an autoclave.
3. In case of solid media tubes, cool them in an incline position (slants)
4. When the medium is cold and solid, inoculate the surface of the medium using pre-sterilized needle. Move the needle gently on the agar surface in a snakelike motion from the butt to the top. In case of broth tubes, inoculate in the liquid media
5. Incubate both culture tubes at 30°C for few days.
6. In case of solid media, scoop out the mass of surface growth in which organism grows and put on clean, dry slide. From liquid broth, place a drop of culture on slide.
7. Observe under microscope.

3.2.4 Observations

In the chart provided:

1. Draw several cells from a typical microscopic field as viewed under each magnification.
2. Give the total magnification for each objective.
3. Observe spores or conidia and their arrangement.

	Bacteria	Yeast	Mould
Drawing of a representative field			
Magnification	----------	----------	----------

3.2.5 Results

Different types of spoilage have been encountered caused by various microorganisms. The type of microorganism proliferating depends on the composition of the material. The different spoilage microorganisms include bacteria, yeasts and moulds that can be observed and identified under a microscope by studying the morphological characteristics. These organisms vary in size, shape, colour, growth habit and mode of reproduction.

3.3 PRECAUTIONS

1. Use clean glass slides for smear preparation.
2. Thick, dense smears should be avoided.
3. Sterilize the inoculating needle before inoculation to avoid contamination.
4. The agar tubes should be properly sterilized.
5. Do not place the cotton plugs on ground during experiment.
6. Carefully view the characteristics of the microorganisms so as to differentiate them correctly.

EXPERIMENT 4 ASEPTIC CULTURE TECHNIQUE

Structure

4.1 INTRODUCTION

In previous experiments you learned that microorganisms thrive pretty much everywhere. It is far too easy to contaminate your lab cultures and experiments with stray microorganisms from the air, the countertop, or your tools. It is also possible to expose your surroundings or yourself to a possible pathogen. In this lab exercise, you will learn to transfer microbiological cultures from one medium to a second sterile medium without contamination of the culture, sterile medium, or the surroundings.

Objectives

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

- know how to handle microorganisms, tubed media, plated media, and inoculating tools such as loops, needles, or swabs etc.;
- learn how to transfer bacteria from test tubes or broth and agar; and
- learn how to transfer bacteria from Petri plates.

4.2 EXPERIMENT

4.2.1 Principle

Aseptic technique is a method that prevents the introduction of unwanted organisms into an environment. In order to protect sterile broth, media, plates, slants etc. from contamination we must practice aseptic i.e. sterile techniques to protect our material from contamination. By using aseptic technique only sterile surface touches other sterile surface and exposure to the non sterile environment is minimized.

Though, observing aseptic technique is the most important instruction for any microbiology experiment, some common circumstances will be discussed in this practical to make you aware of aseptic techniques.

Specific Aseptic Techniques

A) Sterilization of inoculation loop

The inoculation loop is sterilized by passing it at an angle through the flame of a gas burner until the entire length of the wire becomes orange or red hot. In this way all contaminants on the wire are incinerated. Never lay the loop down once it is sterilized or it may again become contaminated. Allow the loop to cool a few seconds to avoid killing the inoculum.

B) Transferring bacteria from broth culture to fresh broth

Requirements

- Bunsen burner.
- Inoculation needle.
- Trypticase Soy Broth cultures of *Bacillus subtilis, Escherichia coli* and *Micrococcus luteus* and *Mycobacterium phlei* – referred to as Tubes A.
- Sterile Trypticase Soy Broth tubes (4 -one for each microorganism) – referred to as Tubes B.
- Glass Marking pen.

Procedure

1. Turn on the Bunsen burner.
2. Vortex culture suspensions of Bacteria given (Tubes A).
3. Place culture suspensions tube near sterile broth tubes (tubes B). Label sterile tubes with name of microorganism and date.
4. Sterilize the inoculation loop as explained above.
5. While holding inoculation loop between thumb and first two fingers of right hand, pick up tube A with left hand and open the cap/cotton plug with last two fingers of right hand.
6. Flame the lip of test tube A.
7. Place the sterile loop into culture A and take loopful of culture.
8. While still holding the inoculum in your right hand, pick up tube B with left hand and open the cap/cotton plug with last two fingers of right hand.
9. Flame the lip of test tube B gently
10. Place the loop containing droplet of culture in tube B and gently swirl it to transfer the microbes into sterile broth..
11. Take out the loop and continue to hold it in your hand.
12. Flame the lip of test tube B gently and replace the cap/plug which should be still in your right hand. Place tube B back in the test tube rack. Like wise plug the tube A and place in a test tube rack.
13. Sterilize the inoculation loop in flame.
14. Repeat the procedure with all bacterial cultures.

Results

1. Draw and describe the growth seen in each of the four broth cultures.

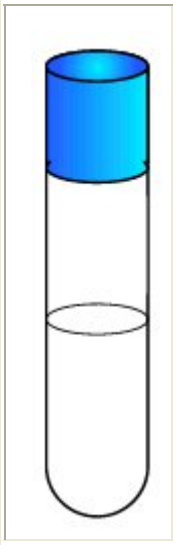
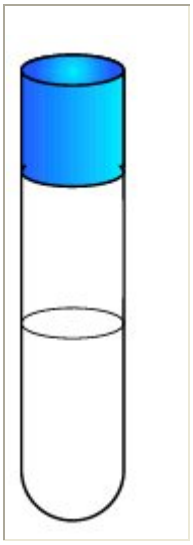
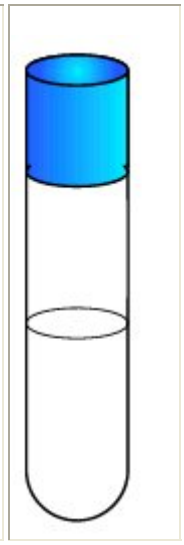
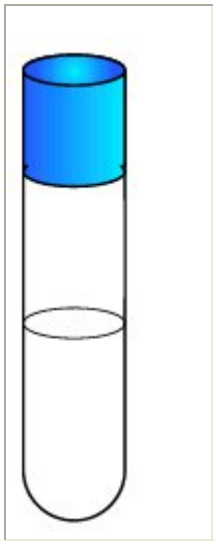
			
Bacillus subtilis Growth =	*Escherichia coli* Growth =	*Micrococcus luteus* Growth =	*Mycobacterium phlei* Growth =

Figure 4.1: Growth of bacterial culture

C) Streaking plating bacteria

a) From broth culture to sterile medium plate
b) From one petridish to fresh sterile medium plate

Requirements

- Bunsen burner.
- Inoculation needle.
- Trypticase Soy Broth cultures of bacteria (*Bacillus subtilis and Escherichia coli*) to be transferred (Tubes A).
- Trypticase Soy Agar plate cultures of bacteria (*Bacillus subtilis and Escherichia coli*) to be transferred.
- Sterile petridish having Trypticase Soy Agar medium (4 no. Two for each bacterium).
- Glass Marking pen.

Procedure

Removing inoculum from a broth culture

1. Label the plates and tubes.
2. Turn on the Bunsen burner.
3. Loosen the top of the bottle/ Tube containing the inoculum.
4. Hold the loop in the right hand.
5. Flame the loop and allow to cool.
6. Lift the bottle/test tube containing the inoculum with the left hand.
7. Remove the lid/cotton wool plug of the bottle/test tube with the little finger of the left hand.

8. Flame the neck of the bottle/test tube.
9. Insert the loop into the culture broth and withdraw. At all times, hold the loop as still as possible.
10. Flame neck of the bottle/test tube.
11. Replace the lid/cotton wool plug on the bottle/test tube using the little finger. Place bottle/test tube on bench.

Removing inoculum from a plate culture

1. Sterilize the inoculating loop in the flame of a gas burner.
2. Lift the lid of the culture plate slightly and stab the loop into the agar away from any growth to cool the loop.
3. Scrape off a small amount of the organisms and close the lid

Transferring the inoculum into a petri plate

1. Partially lift the lid of the Petri dish containing the solid medium.
2. Hold the charged loop parallel with the surface of the agar; smear the inoculum backwards and forwards across a small area of the medium
3. Remove the loop and close the Petri dish.
4. Flame the loop and allow it to cool. Turn the dish through 90º anticlockwise.
5. With the cooled loop streak the plate from area A across the surface of the agar in three parallel lines. Make sure that a small amount of culture is carried over.
6. Remove the loop and close the Petri dish.
7. Flame the loop and allow to cool. Turn the dish through 90º anticlockwise again and streak from B across the surface of the agar in three parallel lines.
8. Remove the loop and close the Petri dish.
9. Flame the loop and allow to cool. Turn the dish through 90º anticlockwise and streak loop across the surface of the agar from C into the centre of the plate
10. Remove the loop and close the Petri dish. Flame the loop.
11. Seal and incubate the plates inoculated with *Bacillus subtilis and* *Escherichia coli* **at 37°C upside down** (lid on the bottom) to prevent condensing water from falling down on the growing colonies and causing them to run together in inverted position.

Results

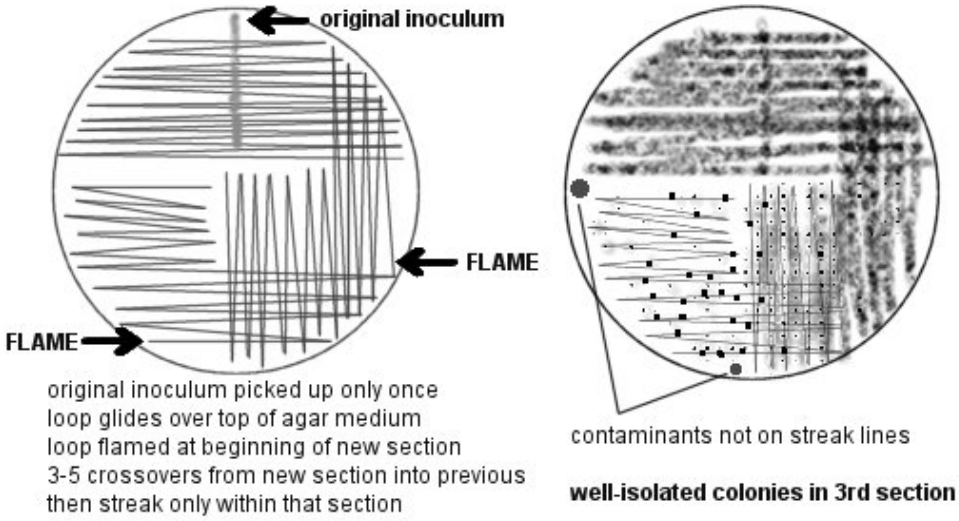


Figure 4.2: Streak plate technique

Expressing results

Bacterial colonies contain millions of cells and exhibit diverse morphologies; however, all isolated colonies produced on streak plates arise from a single bacterial cell. When evaluating colony morphology, use specific terms to describe the shape, elevation, colony margin shape, and surface texture (Figure 4.3). Colony size and colour are also useful features that are noted. All of these characteristics may be useful in the initial identification of unknown bacteria. Colonies that have different morphologies may be considered to contain different bacterial species. However, colonies that appear to be similar in morphology are not always the same bacterial species.

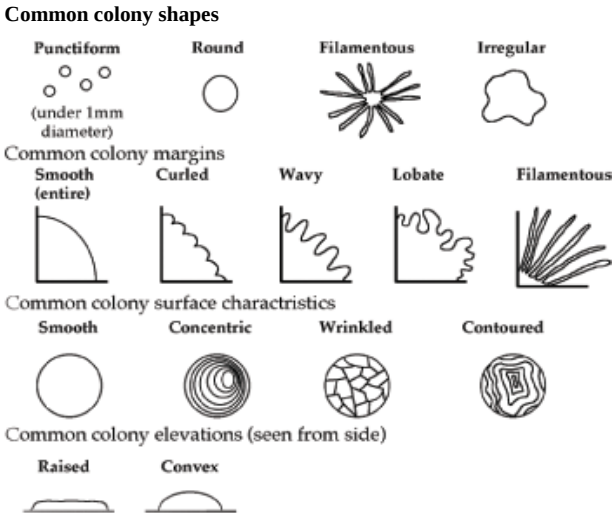


Figure 4.3: Terminology used to describe colony morphology

4.2.2 Observations

Obtain your streak plate from the incubator and visually examine the different regions:

1. Notice a dilution effect as you move from region to region.
2. Look for isolated individual colonies present.
3. Note different types of colony morphologies present.
4. Measure the size (diameter or length) and record the colony colour in Table 1.

Table 1: Colony characteristics of two bacterial colonies isolated using streak plating

Colony Size (mm)	Colour	Shape	Margin	Surface	Elevation
1.					
2.					

4.3 PRECAUTIONS

- Operations must not be started until all requirements are within immediate reach and must be completed as quickly as possible.
- Carry out all microbiological operations in a laminar flow hood.
- Wear gloves and lab coat to protect yourself but also to prevent dry skin and microorganisms from contaminating your samples.
- Use plugs made of non-absorbent cotton wool in test tubes and pipettes to prevent microorganisms from passing in or out and contaminating either the culture or the environment. The cotton wool must remain dry because this filtration property is lost if the cotton wool becomes moist – hence the use of non-absorbent cotton wool.
- For use in test tubes a plug should be properly made to ensure that it can be held comfortably without being dropped and its shape and form are retained while being removed from and returned to a test tube several times.
- Disinfect all surfaces prior to use with a disinfectant solution.
- Swab down the working surface liberally with 70% ethanol.
- Periodically spread a solution of 70% ethanol over the exterior of gloves to minimize contamination. Replace them if torn.
- In case of any spill, spread a solution of 70% alcohol and swab immediately with non-linting wipes.
- Discard gloves after use and do not wear them when entering any other lab area.
- Bring into the work area only those items needed for a particular procedure.
- Leave a wide clear space in the centre of the hood (not just the front edge) to work on. Do not clutter the area to prevent blockage of proper air flow and to minimize turbulence.
- Swab with 70% alcohol all glassware (medium bottles, beakers, etc.) before placing them inside the hood.
- Arrange the work area to have easy access to all of it without having to reach over one item to get at another (especially over an open bottle or flask).
- Use sterile wrapped pipettes and discard them after use into a biohazard waste container.

- Check that the wrapping of the sterile pipette is not broken or damaged.
- Vessels must be open for the minimum amount of time possible and while they are open all work must be done close to the Bunsen burner flame where air currents are drawn upwards.
- Discard any contaminated material immediately.
- Never perform mouth pipetting. Pipetting aids must be used.
- When handling sterile containers with caps or lids, place the cap on its side if it must be laid on the work surface.
- Make sure not to touch the tip of the pipette to the rim of any flask or sterile bottle.
- Clean the work area when finished by wiping with 70% alcohol.

EXPERIMENT 5 VISUAL AND MICROSCOPIC EXAMINATION OF RAW AND PROCESSED PRODUCT

Structure

5.1 INTRODUCTION

Isolation and identification of microbial food contaminants help to understand how infectious agents enter and spread through the food chain. There is a need to estimate the risk that food borne pathogens pose to human health in a national and international context and to identify possible interventions to reduce or eliminate these risks.

Objectives

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

- take visual observations of food samples
- visualize food-borne micro-organisms under the microscope.

5.2 EXPERIMENT

5.2.1 Principle

The potential for food to become contaminated with chemical substances or microorganisms starts from the time it is harvested and continues right through until the time it is eaten. The examination of food samples is one of the most important tasks:

Initial record of specimen as received

- Examine the specimen carefully for information such as to how it was received, condition (frozen, fresh), time, date, mode of delivery and write description of the specimen immediately.
- Examine seals for faults or damage and describe and note the details on the label.
- Weigh/ Measure the specimen as received and prior to opening.

Odour and taste

- Smell the food. The odour of a food can give clues to the nature of the complaint (volatile substances, deterioration, chemical taints etc.) Food may be required to be tasted CAREFULLY at this point.

Spoilage

- Visually observe spoilage by turbidity, gas production, bubbling etc.

Mouldy food

- Document a full description of the affected areas recording the types of colonies present, their colours and their textures. Measure the area(s) of suspect mould as soon as possible and in three dimensions if applicable. Ensure that the dimensions and numbers of individual colonies are noted.

Microscopic Examination of Foods

Principle

Microscopes are instruments that are capable of producing a magnified image of a small objects including microorganisms. In a food microbiology laboratory Compound Microscopes are most commonly used. These microscopes are light illuminated. They are used in observation and description of the microscopic morphology of bacteria, fungi, parasites and host cells in various stained and unstained preparations.

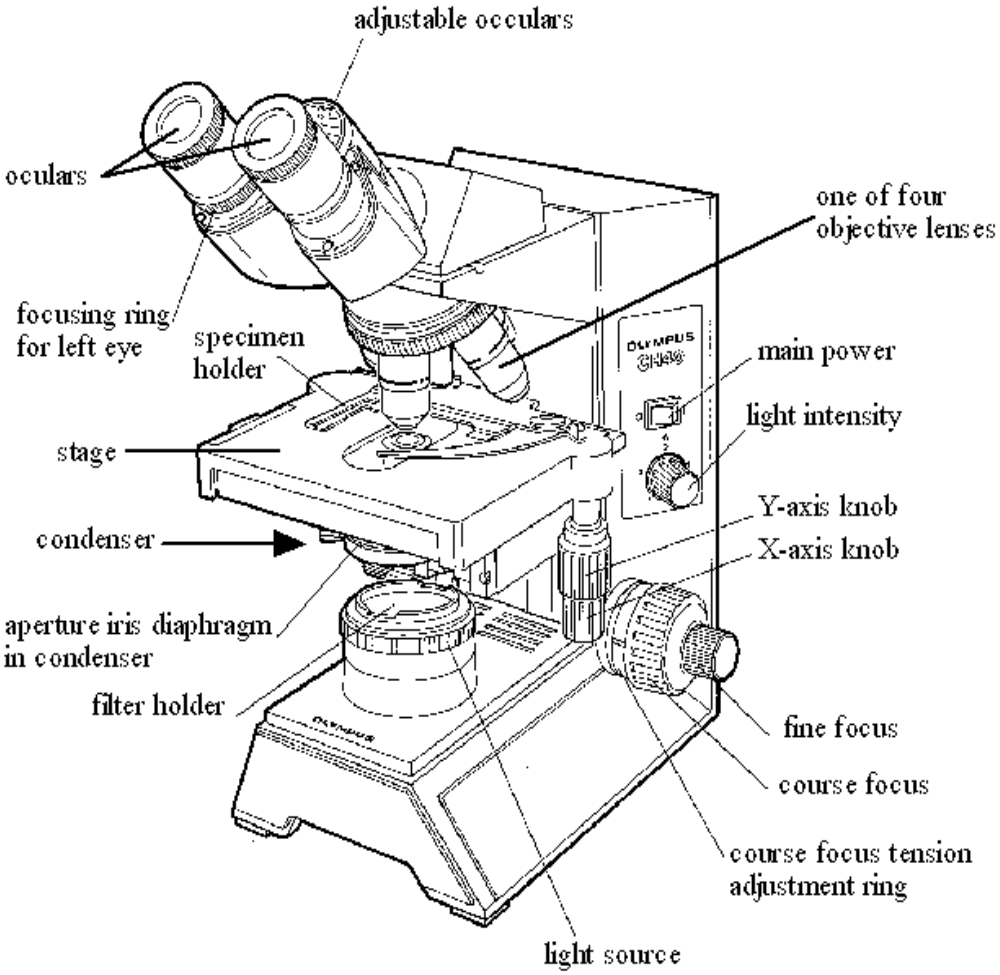


Figure 5.1: Labelled diagram of a compound microscope

Procedure to Focus the Specimen in Microscope

1. Obtain a prepared slide of contaminated food/ isolated microorganism. Mount the slide onto the stage of the microscope.
2. Start with the lowest power objective in place. Using the course adjustment knob, move the objective lens to its lowest point. Look through the ocular and focus upward with the coarse adjustment until an image comes into view. Use the fine adjustment to obtain maximum clarity. From this point on, do not use the coarse adjustment; doing so can result in damage to the lens, slide or both. Adjust the iris to allow enough light for maximum visibility and contrast. Usually, this will be about half the maximum iris opening. Too much light can wash out the details of the image.
3. Move the slide to a point of interest. Move the next objective lens into place and adjust the fine focusing knob, and adjust the iris as necessary. Repeat this step with the highest power, non-oil lens.
4. Note that as the power of the objective lens increases, the distance between the objective and the specimen (working distance) decreases. Also, as magnification increases, the field of view (visible area) and depth of field/focus (visible thickness) decrease. Moving the fine adjustment up and down allows viewing of other areas along the depth of thickness of the specimen).
5. To use the oil-immersion lens, move the turret halfway between the high-power air (non-oil) lens and the oil lens. Place a drop of immersion oil directly on the slide. Move the oil-immersion lens into place and adjust the fine focusing knob. Adjust the iris as necessary. Make sure that the immersion oil does not get on the air lenses. Make note of the differences and similarities between the organisms.
6. After using the oil lens for a specimen, wipe the lens with a piece of lens paper. Do not use anything but lens paper to clean microscope lenses. Usually, lens-cleaning fluids are not necessary unless the lens is exceptionally dirty.

For Getting the Best Possible Image

1. Use lens tissue, to clean the ocular and objective lenses; do not use any other kind of paper. You may also need to clean the slide.
2. Always begin to focus the microscope with the low power, coarse focusing knob.
3. For best viewing at high power, white light is essential. The higher the power of the objective lens, the less will be the depth of field.

Microorganism's Morphology Using the Microscope

Moulds

Mould mycelium and spores can be observed in unstained wet mounts at magnifications of x100 although direct observations of "mouldy" material through the lid of a Petri dish or specimen jar at lower magnifications with the plate microscope are also informative (but keep the lid on!). Routine

identification of moulds is based entirely on the appearance of colonies to the naked eye and of the mycelium and spores in microscopical preparations.

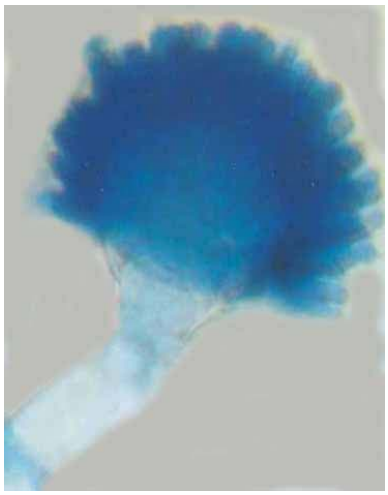


Figure 5.2: *Aspergillus sp.* under microscope

Yeast

Yeast can be seen in unstained wet mounts at magnifications ×100.

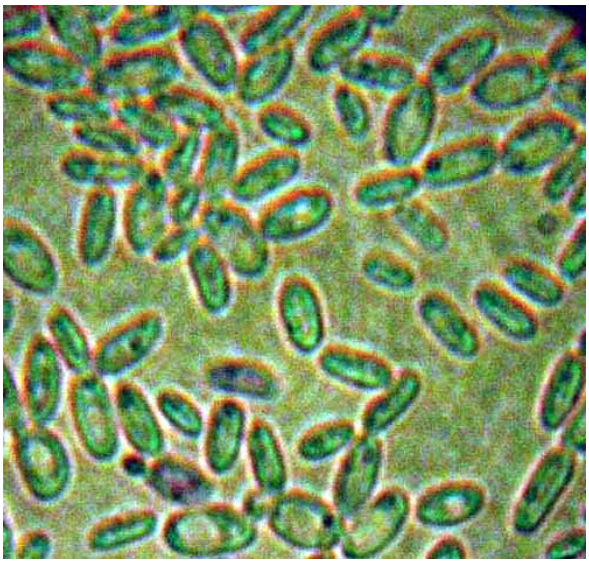


Figure 5.3: Yeast under microscope

Bacteria

Bacteria are much smaller and can be seen unstained at ×400 but only if the microscope is properly set up and all that is of interest is whether or not they are motile. A magnification of ×1000 and the use of an oil immersion objective lens for observing stained preparations are necessary for seeing their characteristic shapes and arrangements. If there is doubt that a food has caused food poisoning or has undergone microbial spoilage, the original product or a low serial dilution of it should be used to prepare a slide for direct microscopic examination. The Gram stain reaction and cellular morphology of the bacteria on the slide may indicate the need for other types of examination. A microscopic examination must be made, even though the food may have undergone heat treatment and the microorganisms involved may no longer be viable.

5.2.2 Requirements

1. Glass slides, 25 x 75 mm, with etched portion for labelling; 1 slide for each blended food sample (10^{-1} dilution)
2. Wire loop, 3-4 mm, platinum-iridium or nichrome, gauge No. 24 or 26
3. Gram stain reagents

Hucker's crystal violet

Solution A

Crystal violet (90% dye content) 2 g

Ethanol, 95% 20 ml

Solution B

Ammonium oxalate 0.8 g

Distilled water 80 ml

Mix solutions A and B. Store 24 h and filter through coarse filter paper.

Gram's iodine

Iodine 1 g

Potassium iodide (KI) 2 g

Distilled water 300 ml

Hucker's counterstain (stock solution)

Safranine O (certified) 2.5

Ethanol, 95% 100 ml

Working solution: Add 10 ml stock solution to 90 ml distilled water.

1. Compound Microscope, with oil immersion objective lens (95-100X) and 10X ocular
2. Immersion oil
3. Methanol
4. Xylene

5.2.3 Procedure

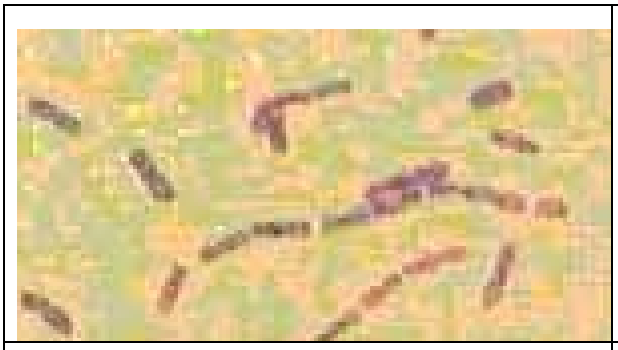
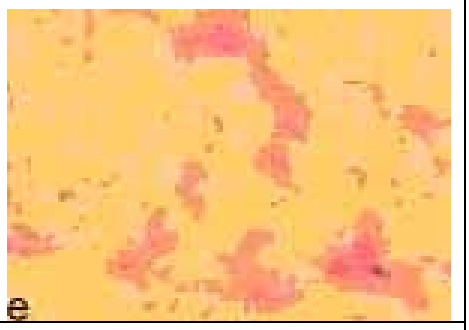
1. Prepare film of blended food sample (10^{-1} dilution).
2. Air-dry films and fix with moderate heat by passing films rapidly over Bunsen or Fisher burner flame 3 or 4 times. Alternatively, air-dry films and fix with methanol 1-2 min, drain excess methanol and flame or air-dry (this is particularly helpful for foods with a high sugar content).
3. Cool to room temperature before staining.
4. De-fat films of food with high fat content by immersing films in xylene 1-2 min; then drain, wash in methanol, drain, and dry.
5. Stain film by Gram-staining procedure.

Procedure for gram staining

1. Fix air-dried films of food sample in moderate heat. Stain films 1 min with crystal violet-ammonium oxalate solution.
2. Wash briefly in tap water and drain. Apply Gram's iodine for 1 min. Wash in tap water and drain.
3. Decolorize with 95% ethanol until blue color is no longer released (about 30 s). Alternatively, flood slides with ethanol, pour off immediately, and reflood with ethanol for 10 s.
4. Wash briefly with water, drain, and apply Hucker's counterstain (safranine solution) for 10-30 sec. Wash briefly with water, drain, blot or air-dry, and examine.
5. Examine under oil immersion and 10X ocular; adjust lighting systems to Koehlor illumination.
6. Examine at least 10 fields of each film, noting predominant types of organisms, especially clostridial forms, Gram-positive cocci, and Gram-negative bacilli.

5.2.4 Observations

- Observe the bacteria under the microscope.

	
Gram positive bacterium	Gram negative bacterium

- Record the Results in table given below.

Table 1: Gram characteristic, size, shape of two bacterial as determined following Gram staining and observation using a compound microscope

Bacterium	Gram Reaction	Cell Size (µm)	Cell Shape
Unknown 1			
Unknown 2			

5.2.5 Results

- Large numbers of Gram-positive cocci on the slide may indicate the presence of staphylococcal enterotoxin, which is not destroyed by the heat treatments that destroy enterotoxigenic *Staphylococcus aureus* strains.

- Large numbers of sporeforming, Gram-positive rods in a frozen food specimen may indicate the presence of *Clostridium perfringens*, an organism that is sensitive to low temperatures. Other Gram-positive, sporeforming rods such as *Clostridium botulinum* or *Bacillus cereus* may also be present in the food.
- When the microscopic examination of suspect food discloses the presence of many Gram-negative rods, consider the symptoms and incubation periods reported for the illness under investigation and select the specific examination method for isolating one or more of the following genera: *Salmonella*, *Shigella*, *Escherichia*, *Yersinia*, *Vibrio*, or *Campylobacter*.

EXPERIMENT 6 ENUMERATION OF BACTERIA BY DILUTION AND PLATING

Structure

6.1 INTRODUCTION

The ability of microorganisms to grow and reproduce in food products is well known. Microorganisms may cause spoilage of the food product. Because of their very small size, counting the number of bacteria in a food sample can be difficult. Although direct counts are possible with a microscope, they require a lot of time and expertise. An easier method is to spread bacteria over a wide area (i.e. nutrient agar plate) and count the number of colonies that grow. If the bacteria are spread out enough, each bacterial cell in the original sample should produce a single colony. Usually, bacterial samples must be diluted considerably to obtain reasonable counts.

Objectives

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

- enumerate bacteria in food samples; and
- isolate pure colonies of bacteria.

6.2 EXPERIMENT

6.2.1 Principle

Since bacterial cell numbers are usually very high in your original sample, plating out this sample in an undiluted fashion would just lead to the creation of a bacterial lawn (a smear of many, many individual bacteria colonies that are all growing next to or on top of one another). Bacterial cell numbers need to be reduced, which is done by repeatedly diluting the amount of bacteria you have in your sample. A small amount of bacteria sample is mixed with a diluent solution (such sterile water or nutrient broth), and then successive dilutions are made. A small amount of each of the diluted bacteria samples is then spread onto an agar plate. The numbers of bacteria colonies that grow on each plate are counted. By working backwards using multiplication with the "dilution factor" (the number of times that you have diluted the bacteria sample with the diluent solution), you will be able to make a determination of the numbers of bacteria in your original sample.

For example, 10,000,000 cell per ml diluted to 100 cells per ml. It is virtually impossible to count 10,000,000 cells on the surface of the agar. However, it is much easier if we dilute the sample and only have to count 300 cells. Diluting is performed in increments because we must obtain a plate with between 30 and 300 colonies (for statistical purposes). Figure 6.1 shows how isolated colonies should look like.

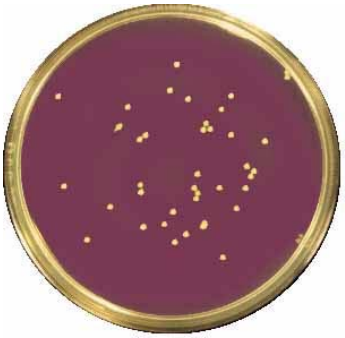


Figure 6.1: Bacterial colonies separated by spread plate method

6.2.2 Requirements

- Nutrient agar plates (6)
- Large sterile tubes (2)
- Tubes with 9 ml of sterile nutrient broth (11)
- Sterile transfer pipettes
- Sterile sticks (2)
- Micropipettor
- Bacteria spreader
- 70% alcohol
- Food samples (say Sample A has been stored in a refrigerator for 4 days;
- Sample B has been stored frozen)

6.2.3 Procedure

DAY 1

1. Label 9 dilution broth tubes as follows:

   A 10^{-2}, A 10^{-3}, A 10^{-4}, A 10^{-5}, A 10^{-6}, A 10^{-7},
   B 10^{-2}, B 10^{-3}, B 10^{-4}

2. Label 6 agar plates as follows:

   A 10^{-6}, A 10^{-7}, A 10^{-8}
   B 10^{-3}, B 10^{-4}, B 10^{-5}

3. Label one large sterile tube A and the other large sterile tube B. Weigh aseptically 1 gram of sample A, and place it in sterile tube A. Add the contents of one tube of dilution broth to the food sample, and shake the sample until the suspension appears fairly uniform. Repeat this with sample B and sterile tube B.

4. Serial Dilutions

   Precaution: You must use a new sterile pipette for each of the dilution steps.

Use a sterile pipette to transfer 1 ml of the suspension from large tube A to the culture tube labeled A 10^{-2}. Mix the contents thoroughly by pipeting up and down several times. Use a new pipette to transfer 1 ml from tube A 10^{-2} to tube A 10^{-3} and mix thoroughly as before. Continue this series of dilutions into tubes A 10^{-4}, A 10^{-5}, A 10^{-6} and A 10^{-7}.

Repeat this series of dilution using sample B. Transfer 1 ml of suspension from large tube B into tube B 10^{-2} and mix thoroughly. Serially transfer, as before, into tubes B 10^{-3} and B 10^{-4}.

5. Plating bacteria

   Use a micropipettor to withdraw 0.1 ml of liquid from tube A 10^{-5} and place it onto the surface of the agar plate labeled A 10^{-6}. (NOTE: Plating 0.1 ml of a 10^{-5} dilution will give you the same number of colonies as plating 1 ml of a 10^{-6} dilution; the agar plate cannot absorb 1 ml of liquid, so the smaller volume is used.)

   Sterilize the bacterial spreader by dipping it into a beaker of alcohol. Remove and shake off the excess. Carefully run the spreader through the flame of a Bunsen burner and allow the alcohol to burn off. Cool the spreader by holding it against the condensation on the inside of the petri dish lid. Gently spread the liquid culture onto the surface of the agar by moving the spreader in a circular manner while rotating the plate. This will ensure an even distribution of bacteria.

6. Repeat step 5 with the remainder of the A cultures:

   Spread 0.1 ml from culture tube A 10^{-6} onto plate A 10^{-7}
   Spread 0.1 ml from culture tube A 10^{-7} onto plate A 10^{-8}

7. Repeat step 5 with the B cultures:

   Spread 0.1 ml from culture tube B 10^{-2} onto plate B 10^{-3}
   Spread 0.1 ml from culture tube B 10^{-3} onto plate B 10^{-4}
   Spread 0.1 ml from culture tube B 10^{-4} onto plate B 10^{-5}

8. Allow plates to absorb the cultures, then turn plates upside-down and incubate overnight at 37° C.

Precautions

1. Before plating, be sure to label each plate with its dilution, date and "food".

2. Mix the samples thoroughly before plating.

3. After pipetting the correct amount of sample in each plate, spread the sample with a bactispreader evenly over the entire surface of the agar.

4. Remember to use aseptic technique. Invert plates. Incubate at 37°C.

6.2.4 Observations

On DAY 2, after incubating the plates, count the colonies on the plate. Each colony represents one cell initially plated. For statistical purposes, pick a plate with between 30 and 300 colonies.

PLATE # COLONIES	PLATE # COLONIES
A 10^{-6}	B 10^{-3}
A 10^{-7}	B 10^{-4}
A 10^{-8}	B 10^{-5}
Bacterial count in sample A (refrigerated food):	Bacterial count in sample B (food):

6.2.5 Calculations

Determine the number of cells/gram in the original sample of food by multiplying the number of colonies on a plate by the dilution factor of that plate.

Calculations dilutions made	**X**	**amount inoculated**	**=**	**"plated dilution"**
dilution factor (simply the inverse of the plated dilution)	X	# colonies	=	# CFUs/ml (or gram) of the original undiluted sample

Example

If a plate labelled 10^{-7} has 87 colonies, then the sample has $87 \times 10^{7} = 8.7 \times 10^{8}$ colonies per gram.

6.2.6 Result

Find the average number of cells/g by adding the results from all of your plates and dividing by the number of plates.

ANNEXURE-1

Composition of different media used for microbial study

1. *Standard Plate Count Agar (SPCA)*

Tryptone	-	5.0g
Yeast extract	-	2.5g
D-glucose	-	5.0g
Agar	-	15.0g
Distilled water	-	1000ml
pH	-	7.0

2. *Nutrient Agar for Bacterial count*

Beef extract	-	3.0g
Peptone	-	5.0g
Agar	-	15.0g
Distilled water	-	1000 ml
pH	-	7.0

3. *Potato Dextrose Agar for Fungal count*

Potato, peeled and diced	-	200g
D-glucose	-	20g
Agar	-	15.0g

Boil 200g of peeled and diced potato for 1 hr in a litre of water. Filter and make up the volume to 1litre and add rest of the constituents.

4. *Violet Red Bile Agar (VRBA) for coliform count*

Yeast Extract	-	3.0g
Peptone	-	7.0g
Sodium Chloride	-	5.0g
Bile Salts	-	1.5g
Lactose	-	10.0g
Neutral red	-	0.03g
Crystal violet	-	0.002g
Agar	-	13.0g
Distilled water	-	1000ml
pH	-	7.4