UNIT 3 OCCURRENCE AND GROWTH OF MICROORGANISMS IN FOOD

Structure

3.1 INTRODUCTION

In the previous unit, we learnt about the various microorganisms in the nature – bacteria, moulds, yeasts and fungi – which are of interest to the food scientist. In this unit, we will try to understand what types of microorganisms are present in air, water and the food itself. How they enter the food and what are the factors responsible for their growth and proliferation. Finally, we will study about the methods which are used to control or destroy these microorganisms in food.

Objectives

After studying this unit, you will be able to:

- enumerate the major reservoir or the support system for microbial growth,
- describe the sources of food contamination,
- discuss conditions conducive for the growth of microorganisms, and
- explain the methods to control or destroy the microorganisms in food.

3.2 MICROBIOLOGY OF AIR, WATER AND SOIL

Have you ever wondered what are the major reservoirs or the support systems for microbial growth? Well, these are the ones which we too require for our survival – air, water and food. Let us get to know about these support systems and the organisms they support. We begin our discussion by dealing with the microbiology of air.

A) *Air*

You would realize that air, by nature, does not contain a natural flora of microorganisms. All that comes into air is by accident and is usually present on the suspended solid materials or in moisture droplet. Microorganisms get into the air or dust or a lint or dry soil, droplets of moisture from coughing, sneezing or talking, sporutalins moulds on walls, ceilings, floors, foods and ingredients. Thus, the air of a dairy plant may contain bacteriophages or at least the bacterial cultures used in the plant. The microorganisms in air have no opportunity for growth, but they merely persist on air and the kinds which are most resistant to desiccation, will live the largest. Mould spores, because of their small size, are resistant to drying and are usually present in the air. Cocci are more numerously found than rod shaped bacteria. Yeasts are also found more in air. It is obvious that dust or sprays of various materials are carried up into the air, the microorganism's characteristic of those suspended materials will be present.

Now, we come on to another important component of environment which is crucial for the growth of microorganisms i.e., water.

B) *Water*

Unlike air, natural waters contain their natural flora, as well as, microorganisms from soil and possibly from animals and sewage. The kinds of bacteria in natural water are chiefly species of *Pseudomonas, Chromobacterium, Proteus, Micrococcus, Bacillus, Streptococcus, Enterobacter* and *Escherichia.* The microbial load of any water depends upon, whether it is stream water, stored water or ground water. Stored water will have more number of microbes than ground water.

What about the microorganisms found in the soil?

C) *Soil*

You would realize that the soil contains greatest varieties of microorganisms. It actually serves as a medium for growth and development of various microorganisms. In fact, if microbiologists want to search for new kinds of microorganism, they usually turn to soil. The soil is the most important source of heat-resistant spore forming bacteria. Important microorganisms associated with soil include *Bacillus, Clostridium, Enterobacter, Escherichia, Micrococcus, Alcaligenes, Flavobacterium, Chromobacterium, Pseudomonas, Proteus, Streptococcus, Leuconostoc, Acetobacteria* and *Actinomycetes.*

Along with air, water and soil, food too is an important reservoir of microbial growth. Which are the microorganisms found in food? We will learn about sources of food contamination in the next section.

3.3 SOURCES OF FOOD CONTAMINATION

All plants and animals have a natural microflora associated with them. For example, the plants have a natural microflora associated with the surface of roots, stems, leaves etc. The animals have a natural microflora associated with the skin, the gut content and external openings e.g. the mouth. These microflora are one of the sources of microorganisms associated with spoilage. Adding on to this, is the contamination from other sources such as water, soil, pests etc. as illustrated in Figure 3.1.

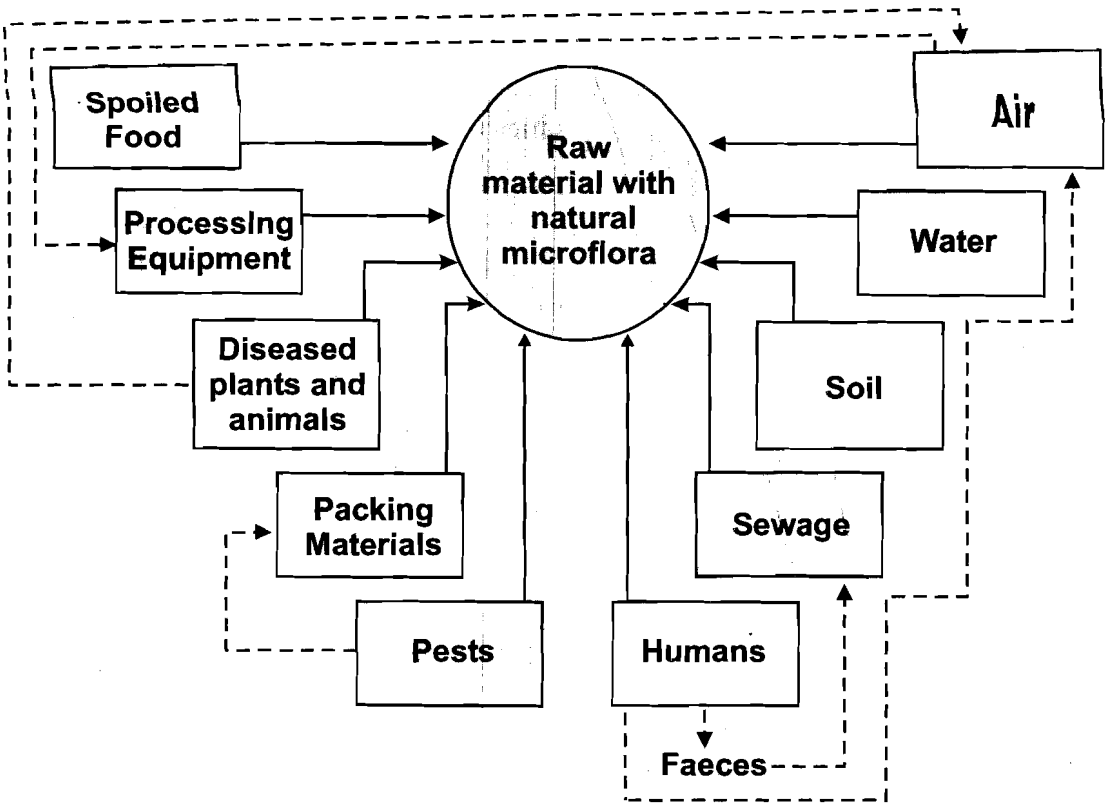

Figure 3.1: Sources of contamination of food

Equipment can be a source of contamination. It can be contaminated during production and also while it is not being used and most important, if not cleaned regularly and thoroughly. Other than the foreign objects, the most common source of microbial contamination in food are the employees i.e. the humans. The hand, hair, nose and mouth carry microorganisms that can be transferred to the food during processing, preparation, packaging and service by touching, breathing coughing etc. Further, water used for cleaning and as an ingredient in many processed food, if not clean/ pure can contaminate food. Microorganisms in air can also contaminate food during processing, preparation etc. Insects and rodents such as mice, cockroaches etc. carry dirt and disease with their feet, fur and faeces. They transfer dirt from garbage dumps and sewers to food or food processing and food service area, thus being a source of contamination. Finally, raw untreated sewage carries high microbial load and may contaminate water, food or equipment through faulty plumbing or otherwise.

So we have learnt about the various sources of contamination. For your information, the type of microorganisms found in each food depends upon the microbial ecology (relationship between microbes and their surrounds), water activity (measure of 'free' water in a food sample, as opposed to 'bound' water) and composition. We will learn more about water activity and other factors affecting microbial growth later in this unit. First, let us look at the type of microorganisms found in selected foods. We shall
in with milk, which you also know is one of the foods which is considered to be
ally wholesome and a highly perishable commodity.

y of milk

for the growth of microorganisms. Do you know why? Yes,
ater, protein and sugar content, it becomes an ideal
rganisms. *Escherichia coli, Bacillus subtilis,*
Alkaligenes are the commonly found bacteria
nd in milk are *Cospora lactis* and other
organisms found in milk also depends

upon the cleanliness of udder, utensils in which the milk is collected, the sanitary conditions of the dairy farm and the personal hygiene of food handlers.

B) *Microbiology of meat*

Like milk, meat is also an ideal medium for microbial growth. Being an animal tissue, it harbours various microorganisms. Though originally, it was thought that the tissues of healthy animals do not contain any microorganism. But analysis of many samples indicated the presence of various types of bacteria. *Micrococci* are normally present and *Salmonella* and *Escherichia coli* are often found. Besides these microorganisms, meat can carry bacteria like *Anthrax, Bacillus tuberculis* and parasites like *Cylicercus bovis, Cylicercus cellulosi* and *Trichinella spiralis.*

C) *Microbiology of vegetables and their products*

All of us are well aware that vegetables are grown in soil, which, we already know, are a major support system for the microbial growth. Vegetables being near and in the ground may carry heterogeneous flora of microorganisms. Microorganisms are transmitted to vegetables if the soil or water is contaminated. *Clostridium, Bacillus* and *Escherichia coli* are the common organisms found in vegetables. Their presence is mostly superficial and not internalised. *Salmonella paratyphi, Shigella dysenteriae, microspine chlorae* are also found in vegetables.

D) *Microbiology of fruits*

Fresh fruits, like vegetables, are in a close vicinity of ground and hence may have varied flora of microorganisms. Nature has given the protective covering to the fruit, but if they are injured, microorganisms enter the fruits. Presence of acid and sugar makes them ideal medium for yeasts and moulds. Common moulds responsible for rotting of fruits include *Aspergillus* and *Penicillium.*

Check Your Progress Exercise 1

1) State whether the following statements are True or False:

   a) Microorganisms in air can sustain life and grow.

   b) The microbial load of water varies with type of water

   c) Of all the three sources of microbes, water is the important source of bacteria.

2) List a few bacteria present in:

a)	Water	.....
b)	Soil	.....

3) What are the common sources of food contamination.

   .....

4) Give the names of at least three microorganisms found in:

a)	Milk	.....
b)	Meat	.....
c)	Vegetables	.....
d)	Fruits	.....

So far we have learnt about the microbiology of air, water, soil and food i.e. the microorganisms found or transmitted through these agents. Have you ever wondered what factors influence the growth of the microorganisms in these mediums? The next section presents an in-depth review of all the factors affecting growth of microorganisms.

3.4 FACTORS AFFECTING THE GROWTH OF MICROORGANISMS IN FOOD

In this section, we will focus on the several important factors which have a bearing on the growth of bacteria. The principal influencing factors include nutrition, oxygen, temperature, hydrogen ion concentration (pH), moisture, osmotic pressure, light and the presence of inhibitory substances like chemicals. Although each of the factors mentioned limits the growth of bacteria, the growth depends more on the combined effects of these factors. Let us get to know about these factors starting with the most important one i.e. nutrition.

3.4.1 Nutrition

Food is food to humans and microbes – be it carbohydrates, proteins or fats. The nutritional requirements of bacteria, however, differ from species to species. The nutrition is required by bacteria not only as a source of energy but also for manufacturing cellular components. The majority of the bacterial species use naturally-occurring organic materials viz., carbohydrates, proteins and fats as a source of energy and the essential elements like carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus. Other elements like iron, magnesium, potassium are required in minute quantities.

Generally, based on the nutritional requirements, the bacteria can be divided into two groups, namely, *autotrophic* and *heterotrophic* bacteria. The *autotrophic bacteria's* requirements of carbon are derived from carbon dioxide or from carbonates and the requirement for nitrogen from gaseous nitrogen or nitrites and nitrates. They have the ability to synthesize its other essential requirements from inorganic substances like sodium chloride (NaCl), dipotassium hydrogen phosphate (K_2HPO_4) etc. for growth and survival. From these simple substances they synthesize complex structures like carbohydrates, proteins, vitamins, enzymes etc. The *heterotrophic* bacteria require one or several preformed organic compounds which are readily available for their growth. These requirements range from a single vitamin to several complex organic compounds. By far, the heterotrophic bacteria are the most commonly found type of bacteria and are widely distributed.

Even the autotrophic and heterotrophic bacteria are sub-divided further depending on the mode of deriving the energy source. They are *chemosynthetic* and *photosynthetic*. The *chemosynthetic* bacteria get their energy from the oxidation of inorganic chemical reactions, whereas, the *photosynthetic bacteria* have the ability to get their energy requirement from the sunlight.

So we have seen how microorganisms can differ based on the nutritional requirement they can utilize for energy and also vary in their need for vitamins or accessory food substances. Some like *Staphylococcus aureus* can synthesize a part of the food they need, others like *Pseudomonas* or *Escherichia coli* synthesize all of the factors needed and still others (the lactics and many pathogens) must have them all furnished.

Like nutrition, oxygen is also basic for the growth of microorganisms? Let's read the next sub-section and find out.

3.4.2 Oxygen

The presence of oxygen in the atmosphere is essential for the survival of human beings. Contrary to this, you would realize that there are many microorganisms including bacteria, which have the ability to thrive in the absence of oxygen or free air. Microorganisms have been classified as *aerobic* when they require free oxygen, *anaerobic* when they grow best in the absence of free oxygen, and *facultative* when they grow well either aerobically or anaerobically.

Moulds are aerobic, most yeast grows best aerobically, and bacteria of different kinds may be aerobic, anaerobic or facultative. The bacteria which have the ability to thrive in the absence of oxygen or free air are known as *'strict' or 'obligate anaerobes'*. These organisms die when exposed to air or oxygen. But there are only few obligate anaerobes. Many bacteria are referred thus, because they tolerate extremely low levels of oxygen. There is another category of bacteria, which can survive either in the presence or absence of oxygen. They are known as *'facultative anaerobes'*. The other kinds of bacteria which cannot survive in the absence of oxygen are called *'obligate aerobes'*. There is yet another category of bacteria known as *'micro-aerophiles'*, which requires oxygen for survival albeit at low concentrations than present in air.

The oxygen tension or partial pressure of oxygen about a food and the *oxidation-reduction potential (O-R)* i.e. the reducing and oxidizing power of the food itself, influences the growth of microorganism. The organisms obtain energy from chemical reactions involving either inorganic or organic compounds. This takes place as an oxidation or reduction reaction through a loss or gain of electrons. The compound losing the electrons is known to be 'oxidized' while the compound accepting the electrons is 'reduced'. The compounds vary in their O-R potential i.e., the tendency to give up electrons. The presence or absence of oxygen and the O-R potential of the food itself has a bearing on the type of organism which grows on a particular food. The O-R potential of a system is usually written *Eh* and measured and expressed in terms of millivolts (mV). A high (oxidizing) potential favours aerobes but will permit the growth of facultative organisms and a low (reducing) potential favours anaerobic or facultative organisms.

Let us study about the role of temperature next.

3.4.3 Temperature

Temperature is one of the important factors affecting the process of growth in bacteria, as it has a bearing on chemical reactions. The temperature at which the maximum growth occurs is known as the *optimum temperature*. Based on the temperature at which the maximum growth occurs, the bacteria are divided into three main categories:

1) *Thermophiles:* The species of bacteria which grows rapidly between 45-65°C, although they may grow anywhere between 45-75°C.

2) *Mesophiles:* The species of bacteria which grows rapidly between 20-45°C, where the optimum temperature range is 30-40°C.

3) *Psychrophiles:* The bacterial species which grows rapidly at 0°C (and even below), and have an optimum temperature range of 10-20°C.

Although bacteria have been grouped into three categories based on temperature, there is no certainty that there is no overlapping between the bacterial groups. The division is based on a broad range of species. To illustrate, raw milk held at different temperatures support the initial growth of different bacteria. At temperature near freezing, cold tolerant bacteria, e.g. species of *Pseudomonas* will grow and *Alcaligenes*

are favoured at room temperature. *Streptococcus lactis* and *coliform* bacteria usually predominate at 40° to 45°C. At 55°C, thermophilic bacteria like *Lactobacillus thermophillus* will grow.

Moulds and yeasts, for the most part, do not grow well above 35° to 37°C and therefore, would not be important in foods held at high temperatures. On the other hand, moulds and yeasts grow well at ordinary room temperature and many of them grow fairly well at low temperatures, some even at freezing or slightly below. Therefore, moulds often grow on refrigerated foods.

3.4.4 Moisture Requirement – The Concept of Water Activity

We already know that moisture is one of the important factors for the survival of living species. As such, it has a great influence on them, including microorganisms like bacteria. Water accounts for 80-90% of the total weight of cells. The water requirement of bacteria varies from species to species. Although water is required by bacteria, the growth largely depends upon the available water, which can be utilized for the growth of microorganisms. This is expressed as *water activity* which is defined as the percent equilibrium relative humidity (%ERH) divided by 100. ERH is *an expression of the amount of free water vapour present on a product, both at its surface and within its structure*. Numerically, ERH of a food divided by 100 equals "Water Activity". This can be represented by the following equation:

$$\text{Water Activity } (a_w) = \frac{\text{Equilibrium Relative Humidity}}{100}$$

In definition, water activity, expressed as the percent equilibrium relative humidity (%ERH) divided by 100 is unexciting. *In practice, water activity is really a measure of 'free' water in a food sample, as opposed to 'bound' water.*

Besides water activity, you may come across another concept 'water content' while talking about moisture. You must understand that water activity is different than the water content in foods. *Water content, when referring to a solid material, is an expression of the percentage of the materials weight which is water (both in liquid or gaseous phase), usually referred to as 'percent moisture content'.*

The reason why water activity merits closer attention is that it influences the shelf life of a food product. Water activity affects microorganism survival and reproduction, enzyme and chemical reaction. While temperature, pH and several other factors can influence if and how fast organisms will grow in a product, water activity may be the most important factor in controlling spoilage. We will learn more about this concept later in Unit 11, while talking about food packaging.

The nutrition and temperature have been found to have a bearing on moisture. With an increase in temperature, the available water will be reduced and then the availability of nutrition determines the growth. Most bacteria have been shown to grow well in media with water activity (a_w) between 0.990-0.998. Water activity is an important factor in the control of growth of microorganisms.

3.4.5 Osmotic Pressure

Another important characteristic of a cell is 'osmosis'. You would recall reading about osmosis in the Applied Physiology Course in Unit 8. In this section, let us learn more about cell and how it behaves when placed in water or any other solution.

Do you remember the structure of bacteria? Yes, bacterial cell is contained by a cell membrane, which allows water to pass in and out of the cell. Active bacteria contain an excess of 80% water. When bacteria is placed in a heavy sugar syrup or salt

brine, water in the cells move out through the membrane and into the concentrated syrup or brine containing 30-40% water. This is known as *'osmosis'*. In the Figure 3.2, you can see a simple representation of the process of osmosis, where a membrane separates the solute molecules. Transfer of a liquid solvent through a semipermeable membrane that does not allow dissolved solids (solutes) to pass is osmosis.

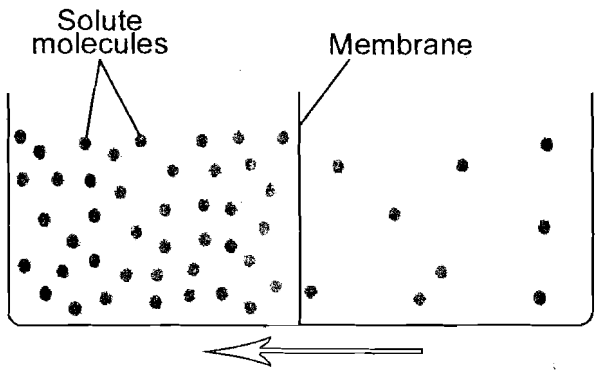

Figure 3.2: Osmosis

The tendency to equalize water concentration inside and outside the cell in this case causes partial dehydration, where the cell shrinks and is called *'plasmolysis'*. Instead, if the bacterial cell is placed in distilled water, the water enters the cell and causes it to burst. This is known as *'plasmoptysis'*. The tendency of cell membrane to allow water to pass from inside to outside or vice versa, is there to maintain an equilibrium between the cell contents and its fluid surroundings. As the hydrostatic pressure causes osmosis, it is known as *osmotic pressure.*

The osmotic pressure is related to the water activity of solutions and foods, solutions high in solute concentrations have a high osmotic pressure and a lower water activity. Dilute solutions are lower in osmotic pressure and have a high water activity.

3.4.6 Hydrogen Ion Concentration – pH

The hydrogen ion concentration has a significant role to play in the growth of bacteria, as every microorganism has a minimal, maximal and an optimum pH at which it can exist and thrive.

The pH, as you may already know, is defined as *the negative logarithm of the reciprocal of the hydrogen ion concentration,* expressed as:

$$pH = -\log \frac{1}{(H^+)}$$

The pure water when ionized contains 10^{-7} moles each of H^+ and OH^-. As there is a balance between H^+ and OH^- ions, the solution (water) is neutral (pH 7). A pH scale has been devised to indicate the pH of various foods. The pH of water being neutral (pH 7), it is the mid point of the scale. The pH scale extends from 0-14. Any substance is known to be alkaline if the pH is above 7. Most of the bacteria prefer a pH near 7 (neutrality), whereas, there are some bacteria which prefer alkaline or acidic medium. In general, yeasts and moulds are more acid tolerant than bacteria.

Finally, let us look at the role of light in the growth of microorganisms.

3.4.7 Light

Although visible light is beneficial to the photosynthetic bacteria, the ultraviolet light (UV) is however harmful to the bacteria. The UV light is absorbed by the nucleic acid present in the cells, which gets denatured and may result in the death of cells.

Due to this property, UV light is used in the sterilization of air, water etc. We will learn more about this later in this unit under methods to control bacteria.

With this, we come to an end on our discussion on factors affecting the growth of microorganisms. These factors are important and you will learn more about their relevance and implication while discussing food safety or food preservation later in this course.

Check Your Progress Exercise 2

1) Fill in the blanks:

   a) ..... and ..... do not grow well above 35°C to 37°C and therefore are not important for foods held at high temperature.

   b) Based on the nutritional requirement, bacteria can be divided into ..... and ..... .

   c) Chemosynthetic bacteria derive their energy from ..... of inorganic chemical reaction while photosynthetic bacteria get energy from ..... .

   d) The temperature at which maximum bacterial growth occurs is ..... temperatures.

   e) Based on temperature, bacteria are divided into....., ... and ..... .

2) What are the factors affecting the growth of microorganisms?

   .....

   .....

   .....

3) Explain the following terms:

   a) Facultative anaerobes

   .....

   .....

   b) Plasmolysis

   .....

   .....

   c) Osmosis

   .....

   .....

   d) pH

   .....

   .....

   e) Water Activity

   .....

   .....

Earlier we learnt about the factors influencing the growth and multiplication of microorganisms. Just like the way these factors favour growth, they can also be manipulated to control the growth or cause destruction of the microorganisms. The next section presents an insight into this interesting aspect of food microbiology.

3.5 CONTROL AND DESTRUCTION OF MICROORGANISMS

Destruction or control of growth of microorganisms is the basis of food preservation. To 'preserve' actually means to keep safe, retain quality and prevent decomposition. Food preservation, as you would realize, *is a process by which certain foods can be preserved from getting spoiled and kept safe for a longer period of time.* The colour, taste and nutritive value of the food are also preserved.

Microorganisms can be destroyed or controlled by the manipulation of the very factors like pH, temperature and water activity, which affect the growth of the microorganisms, using physical or chemical methods. This is the principle behind food preservation. Let us learn about these methods of food preservation by describing the physical methods first.

A) *Physical Methods*

Physical methods include various methods to control and destroy growth of microorganisms such as pickling, fermentation, dehydration, pasteurization, sterilization, canning, irradiation, freezing etc. You will find that all these methods are discussed in a great detail in the Course Principles of Food Science in Units 10, 11 and 12. Here an overview of these methods is presented for you to understand the mechanism and how and which factor(s) is manipulated for the control of microorganisms. The methods include:

- *Pickling:* Pickling is the process of preparing a food by soaking and storing it in a brine (salt) or vinegar solution. Pickling vegetables and fruits with vinegar helps in the prevention of microbial growth. This is due to the food being placed in a low pH solution in which microorganisms cannot grow.

- *Addition of Sugar or Salt:* The addition of large quantities of sugar or salt inhibits the growth of microorganisms by making the water unavailable involving osmosis. Jams, marmalades, jellies and pickles employ this principle. Salted foods and foods with added sugar are also effectively preserved using the same technique, since the water they contain is unavailable for microbial growth. Indeed, cells of microorganisms become plasmolysed when they come in contact with the surface of these foods.

- *Fermentation:* Fermentation is a process by which the living cell is able to obtain energy through the breakdown of glucose and other simple sugar molecules without requiring oxygen. Fermentation, acts as a preservation method by producing an acid, which lowers the pH of the product, converting a perishable food into the one that has a longer shelf life, e.g. fresh milk to cheese. The basis of the fermentation process is the conversion of glucose (sugar) to alcohol or to lactic acid by enzymes and we learnt earlier that at low pH microorganisms cannot grow.

- *Drying:* Control of microorganisms by drying is based on the fact that microorganisms and enzymes need water in order to be active. When the moisture content is reduced in foods, microorganism's growth is retarded. Drying reduces a_w level, weight and bulk of the food and helps in food preservation. The main principle of drying, therefore, is to prevent microorganism activity by reduction

of water. Although some microorganisms are destroyed in drying, it is not *per se* lethal. The earliest method of drying was by simply exposing fresh foods to sunlight until drying is achieved. Fruits such as raisins, prunes, figs and others are dried by this method. The commercial drying methods consist of spray drying, drum drying, tunnel drying, freeze drying etc. You shall learn more about these methods in the Course Principles of Food Science, in Unit 11.

A few food products that are preserved using commercial drying methods are briefly highlighted herewith:

— *Milk:* It is dried as either whole milk or non-fat skim milk. Dehydration is accomplished by either drum-drying or spray-drying.

— *Eggs:* It may be dried as whole egg, yolk or white.

— *Vegetables:* These are generally preserved by freeze drying.

— *Meat:* It is usually cooked before being dehydrated.

In freeze drying, the sample is freezed and water present in the form of ice is removed by sublimation.

- *Temperature:* You have already studied in this unit about temperature as one of the important factors affecting the growth of microorganisms. Here we shall see how an increase or decrease in temperature could help us in controlling and destroying the growth of microbes. We begin with high temperature.

  a) *High Temperature*

  High temperature means any and all temperatures above ambient (room) temperature. In high temperature, there are three categories which are used in controlling or destroying microorganisms: (1) Pasteurization, (2) Canning, and (3) Sterilization.

  1) *Pasteurization:* Pasteurization means *destruction of all pathogenic organisms in food (e.g., milk) or reduction of spoilage organisms in certain foods (e.g., vinegar)* using mild heat.

  You might have seen, as well as, consumed a variety of pasteurized products. Do you know what it is and how is it done? Well, when foods are heated in containers or by other methods to a temperature below 100°C for a definite period, the process is called 'pasteurization'. Generally, the pasteurization process involves heating of the food to temperatures between 60°C and 85°C for a few seconds up to an hour. The food is cooled promptly after the heat treatment. There are two general types of pasteurization: (1) *temporary,* the purpose being either to destroy some pathogenic organism or to control any spoilage organism, and (2*) permanent*, in which the food material is subjected to a low temperature for the necessary length of time to ensure the keeping quality. The containers in this method are generally hermetically sealed. The shelf-life of food products increases by several days and are usually stored in refrigerated conditions. This method is used to preserve fruit juices such as grape juice and fresh milk. It is also safely used in preserving most soft fruits.

  The process of pasteurization originated primarily when the French scientist *Louis Pasteur* (1822-1895) employed this technique for controlling the contamination of wild yeasts and bacteria, which are responsible for spoiling wine, which was later extended to beer. In the later years, the pasteurization was applied to the preservation of fruit juices. Under the acid conditions prevailing in juices, the organisms are destroyed. Hence, preservation is usually permanent.

Milk is pasteurized to destroy pathogenic organisms, reduce total bacterial numbers, extend the storage life and also to inactivate enzymes which can affect milk flavours adversely. Prior to pasteurization of milk, diseases like typhoid, scarlet fever, diphtheria, tuberculosis and brucellosis were common due to the consumption of contaminated milk.

Traditionally, the pasteurization of milk is carried out by Low Temperature Long Time (LTLT) process i.e., holding the milk heated between 63 to 65°C for 30 minutes. In the High Temperature Short Time (HTST) process, the milk is heated rapidly to 71.7°C and held for 15 seconds and immediately cooled to 4°C. The other temperature ranges at different holding period are also adopted. In the Ultra High Temperature (UHT) pasteurization, milk and other dairy products are heated at 132°C for at least 2 seconds. By adopting this process, *Coxiella burnetii,* the organism responsible for "Q" fever, is eliminated. The heat treatment has to be elevated for the dairy products like cheese. The ice cream mix may be heated at 71.1°C for 30 minutes or at 82.2°C for 16 to 20 seconds.

Canning: The term canning is generally applied to foods, more specifically, to the foods preserved by heat processing. It aims to destroy microorganisms and their spores through the application of heat. The term "canning industry" is therefore primarily meant to include the entire range of foods preserved by heat treatment whether packed in tin or aluminium cans or glass or thermostable plastic pouches. The process of canning is also known as *"appertization"* named after a French scientist *Nicolas Appert*, who published the directions for canning process and he is known as "father of canning".

Now how to successfully accomplish or carry out the canning process? Apart from this, are there any factors which influence the canning process? If yes, which are these? Let us find out.

In the process of canning, the temperature used for heat processing varies from 100°C for high acid foods to 123°C for low acid foods. For HTST process, temperatures in the range of 120° to 150°C or higher may have to be used. The process normally gives a better quality product when compared to a product subjected to longer time at lower temperatures.

The heat processing for canning depends on a few essential factors such as pH, initial bacterial load, salt and sugar concentration in the product. The pH of a food to be canned is one of the important factors which determine the temperatures to be used. The pH affects the quality and stability of the food product. The critical pH on which most processes are based is pH 4.5, which is the lowest pH at which *C. botulinum* can grow under normal conditions. With a few exceptions, the heat resistance of the bacteria is more when it is near to the neutral point i.e., pH 7. If the product is acidic in nature, the bacterial spores die.

What are the conditions where the spores can still survive inspite of the process of canning? Pathogenic and thermo-resistant spore formers, that may be associated with the raw materials or ingredients like spices as an initial bacterial load, pose a hazard for canning. So, proper care prior to canning, can eliminate this danger to a great extent.

Conditions for various bacteria differ with regard to salt and sugar concentrations in the product. The bacterial spores display heat resistance up to a salt concentration of 4%. But as the concentration increases, the resistance drops. As far as sugar concentration is concerned, an increase of sugar greatly reduces the heat susceptibility.

3) *Sterilization:* The process involves the use of a combination of high temperatures and time to destroy nearly all microorganisms in food. This process is more severe than pasteurization and can sometimes affect the taste and appearance of the food. Then why is this method used? This method is important as some microorganisms (like *Clostridium botulinum*) can form spores, which have the ability to survive at high temperatures. If the correct temperature is not reached, there is the possibility that the spores will germinate and grow and food poisoning could result. Food products that have been sterilized have a very long shelf period at ambient temperature, e.g. canned ham or long life milk. These types of products would have a 'best before' date mark on the label, which means that the product should be consumed before that date.

4) *Irradiation:* It is *the process of exposing food to carefully controlled amount of ionising energy to destroy and control microorganisms in food.* In fact, food irradiation can produce a wide variety of beneficial effects including delay in the ripening of fruits and vegetables, inhibition of post-harvest sprouting of tubers and bulb crops besides destruction of insects, parasites, bacteria, yeasts and moulds which can cause food spoilage or poisoning. There are three types of treatments that are given to food, which vary in terms of dosage level of radiation. These are:

   a) *Radappertization:* It is the treatment of a food with a dose of irradiation, usually 25 to 45 kgrad to produce a commercially sterile product, similar to canning. This high dose food application is generally used for foods, is meant for immuno-compromised people and astronauts etc.

   b) *Radicidation:* It is the treatment of a food with a dose of irradiation sufficient to reduce the number of viable, non-spore forming, pathogenic bacteria to undetectable levels which is equivalent to pasteurization with heat and the dose is generally less than 10 kgrad.

   c) *Radurization:* It is the treatment of a food with a dose of radiation (<10 kgrad) in sufficient quantities to reduce the number of spoilage microorganisms and to enhance the keeping quality of the foods.

Next, let us learn about use of low temperature as a preservation technique.

b) *Low Temperature*

Like in high temperature, lowering the temperature of a food product too helps to control, as well as, destroy the growth and survival of microorganisms. Let us briefly look at the major low temperature techniques of bacterial control. We shall begin with chilling.

i) *Chilling:* You may have noticed that the refrigerated storage is the most widely practiced methods of controlling microorganisms in perishable foods. When temperatures are lowered below the optimum for growth of a particular organism, it is seen that the log time and generation time increases and growth rate decreases until the temperature approaches the minimum for growth, the cell division ceases.

ii) *Freezing:* Besides chilling, freezing is another method of control of microorganisms. It was mentioned earlier that decreasing the temperature decreases the metabolic activity in organisms and freezing totally arrests the growth of microorganisms. Freezing, hence, is based on the following two principles:

   1) Very low temperatures, which inhibits growth of microorganisms and retards the enzymic and chemical activity, and

2) Formation of ice crystals, which draws available water away from food, therefore, prevents the growth of microorganisms.

There are certain methods of freezing that have been developed in the food industry to speed up the freezing time, as well as, reduce the risk of microbial growth. These are:

— *Blast freezing*: Batches of food are subjected to a constant, steady stream of cold air (– 40°C or lower) in a tunnel or a cabinet.

— *Scraped heat exchange*: In this, the product such as ice-cream is scraped against a cooled surface. This is to reduce the formation of large ice crystals.

— *Cryogenic freezing*: Liquid nitrogen/ CO_2 is sprayed directly onto small food items (prawns, soft fruits). Due to the liquid's extremely low temperatures (–196°C and –78°C respectively), freezing is almost instant.

Fish and fish products, beef and their products, vegetables and ready meals are some of the foods where freezing is used.

- *Modified Atmospheric Packaging:* Modified atmospheric packaging is the enclosure of food in a package in which the atmosphere has been changed by altering the proportion of carbon-dioxide, oxygen, nitrogen, water vapour and trace gases. The process retards microbial and biochemical activity.

Check Your Progress Exercise 3

1) Fill in the blanks:

a) The oldest method of drying is ..... .

b) Production of ..... acid lowers the pH of a fermented product.

c) .....is the process of heating foods to a temperature below 100°C for a definite period, followed by prompt cooling.

d) Some of the beneficial effects of ..... include destruction and control of microorganisms, delay in ripening and inhibition of post harvest sprouting.

e) The three irradiation processes are ....., ... and .....

2) Explain the main principle behind food preservation. What are the two methods used for food preservation?

.....

.....

.....

3) List the different physical methods of preservation.

.....

.....

4) Give the time and temperature ranges of:

a)	LTLT	.....
b)	HTST	.....
c)	UHT	.....

So far we have learnt about the physical methods of preservation, based on the manipulation of one or a combination of factors to control microbial growth. Next, we shall review the chemical methods to control and destroy microorganisms.

B) *Use of Chemicals to Control and Destruct Microorganisms in Foods*

The use of chemicals, as a process of food preservation, has been used since long, ever since man found them by trial and error to be beneficial to him. Sodium chloride or the common salt has been the oldest compound which has been serving the purpose since several centuries. Apart from sodium chloride, other chemicals which were being used include salt peter, slaked lime etc. The salt peter (sodium and potassium nitrate) in combination with common salt was used to impart flavour and keeping quality of meats. All these compounds can be termed as a preservative. Now, then, how would you define a chemical preservative? Let us find out.

A chemical preservative is *a substance which is capable of either inhibiting, retarding or arresting decomposition of foods*. The use of chemicals as preservative has been subjected to criticism due to their indiscriminate use and the likely hazard they pose to the human health. The process of preservation by the addition of chemicals to foods has to be undertaken, keeping in mind the usefulness and limitations in affecting human health.

International organizations, such as the Food and Agriculture Organization (FAO) and World Health Organization (WHO), have defined limits for each chemical to be used as a preservative. The Government of India regulates the use of various additives in foods. The use of chemicals has to be employed, if it is an economical means of preservation and only when other physical means like pasteurization etc. are not possible or other methods of preservation are not available. The preservative should extend the storage life of the product and at the same time, not affect the quality and be an antimicrobial agent. It also should not be converted to other toxic compounds on reaction with the food commodity. We will learn more about chemical preservatives later in this Course in Unit 7. But here we must understand how do these preservatives extend the storage life of the product. Remember, the chemical preservatives serve as antimicrobial agents by acting as growth inhibiting or growth retarding agents or by killing the microorganisms. The action of most chemical agents on microbes include their effectiveness either by their effect on their genetic functions by: (a) interacting with the DNA molecules (b) acting upon the cell wall (c) inhibiting the microbial cell enzymes, and (d) making the essential nutrient unavailable to the microorganisms.

What are the types of chemical preservatives commonly used? Let's find out. There are several food preservatives which have been 'Generally Recognized As Safe' (GRAS) in the USA and for which Acceptable Daily Intake (ADI) have been specified by the FAO/WHO (for details refer to Unit 7, section 7.3). These are widely used in many countries of the world to help prevent the bacterial and fungal contamination of foods. Such chemicals include: Benzoates, sorbates, propionates, acetates, nitrates and nitrites, sulphur dioxide and sulphites, and antibiotics.

A word on each of these preservatives follows:

a) *Benzoates*

Benzoic acid, sodium benzoate and the parahydroxy esters (parabens) are used as preservatives. Benzoic acid and its sodium salt, sodium benzoate, is the widely used preservative when compared to other preservatives. Sodium benzoate is more soluble in water, as such, it is the preferred form.

The antimicrobial activity of benzoate is greater at the lower pH values. Its optimum pH range is between 2.5 to 4.0. Only the undissociated benzoic acid molecules are active. Due to this, the benzoic acid and its salts are effective in high acid food products. These compounds act as inhibiting agents for some moulds and yeasts in acid fruit products like jams, jellies, fruit juices etc. The two esters, methyl paraben and propyl paraben are also used extensively, as they are effective at a higher pH level when compared to benzoates. This is due to the esterification of carboxyl group, which makes the undissociated molecule being available over a wider pH range.

b) *Sorbates*

Sorbic acid is an unsaturated carboxylic acid whose salts as sodium, calcium or potassium are used in foods upto 0.2%. As in the case of benzoates, the salts of sorbic acid are fungal inhibitors and are effective below pH 6.0 and comparatively act better between pH 4.0 to 6.0. Sorbic acid is widely used in bakery products, fruit juices, jams, pickles and especially cheese and cheese products. It effectively controls the cheese spoilage bacteria and being non-toxic, it does not impart off-flavours. It is effective at the entire pH range of cheese and does not affect the bacterial ripening process. It is also applied to the bread wrapper, which eliminates the mouldy appearance in bread.

c) *Propionates*

Propionic acid is formed from lactic acid or lactates, as a result of the bacterial action during the manufacture of Swiss cheese. The characteristic 'eye' formation in Swiss cheese is due to the gas formation by the bacteria. It serves as a developed preservative up to 1% and prevents the growth of moulds. The calcium and sodium propionates are extensively used in the bakery products to prevent the 'rop' by *B. mesentericus*. They do not have much effect on yeasts and bacteria. The inhibition of moulds is best between the pH 3.5 to 4.5.

d) *Acetates*

Acetic acid is an approved GRAS food preservative. This is a widely used preservative due to being freely available and cheap, when compared to other products. It is effective against bacteria. As a principal component of vinegar, it is used in many foods. The derivatives of acetic acid, dehydroacetic acid and sodium diacetate are being used as preservatives. Among them, dehydroacetic acid is used to impregnate wrappers for cheese in inhibiting growth of moulds and sodium diacetate is used for inhibiting moulds in bread. These two are also GRAS preservatives. The action, like in benzoates, propionates and sorbates, increases with a decrease in pH.

e) *Nitrates and Nitrites*

The use of nitrates and nitrites has been widely used in the curing of meat. The reduction of nitrates by bacteria produces nitrites under acid conditions created by meat. Recent research has pointed out that nitrites can react with secondary and tertiary amines to form nitrosoamines which have been known to be carcinogenic to humans. This is alarming. The use of nitrites as an inhibitory agent against *Clostridium botulinum* in meat products is well established.

f) *Sulphur dioxide and sulphites*

Sulphur dioxide as a preservative in wine preparation has been in use since ancient times. It is also used in other foods, like fruits and vegetables, for preventing the microbial growth. Sulphur dioxide is effective in inhibiting bacterial and mould growth when compared to yeasts. It is also used in combination with sodium benzoates in fruit preparations. Apart from sulphur dioxide, other sulphites, sodium sulphite, potassium sulphide and sodium metabisulphite etc. are also used as preservatives, as they act more or less similarly in inhibiting microbial growth. The inhibition of microbial growth is through the inhibition of certain enzyme systems and other biochemical reactions and is also pH dependent. The sulphur dioxide forms sulphurous acid (H_2SO_3) when it combines with water and is effective at the lower pH values.

You must have heard about antibiotics and its role in diseases. What are these and how are these produced? Let's find out.

g) *Antibiotics*

The antibiotics are *the chemicals produced by microorganisms which are able to destroy or inhibit the growth of other microorganisms*. The therapeutic use of antibiotics in bacterial diseases is well established. This led to the thought of using antibiotics for the prevention of microbial spoilage of foods. Several antibiotics were tested as preservatives but their use has been discouraged, since it was observed that antibiotic resistant microorganisms were developing due to regular use. Only nisin produced by certain strains of milk souring organism, *Streptococcus lactis* is permitted to be used as a preservative in foods in several countries, including India.

With this, we come to an end to our discussion on methods used to control microbial growth. We hope this information equipped you in understanding the role of different factors influencing both the growth and survival of microorganisms in food.

Check Your Progress Exercise 4

1) Fill in the blanks:

   a) ..... and ..... combination is used to impart flavour and keeping quality of meats.

   b) .....preservative is a substance which is capable of either inhibiting, retarding or arresting decomposition of foods.

   c) Preservatives which have been specified as ..... can be safely used.

   d) The optimum pH range of benzoate, to act as a preservative is.....

   e) Nitrate acts as an inhibitory agent against ..... bacteria in meats.

2) Explain how chemical preservatives act as antimicrobial agents.

   .....

   .....

   .....

3) List a few GRAS chemicals that are widely used.

   .....

   .....

4) Mention the chemicals that are used to preserve the following food items.

	Food Items	Preservative
a)	Jams and Jellies	.....
b)	Swiss Cheese	.....
c)	Breads	.....
d)	Meat	.....
e)	Wine	.....

3.6 LET US SUM UP

In this unit we studied the microbiology of air, water and soil which act as physical sources of a variety of microbes. The various sources of food contamination were also discussed in this unit. The major focus of the unit was on the factors affecting the growth of microorganisms which included pH, water activity, nutrition, OR potential etc.

Further, the methods to control and destroy and growth and development of microorganisms in foods were dealt with. These involved among the physical methods, the use of altered temperatures (high and low), drying, fermentation and chemical preservatives such as sorbates, propionates, benzoates etc. The use of antibiotics to prevent microbial spoilage of foods was also highlighted.

3.7 GLOSSARY

Antibiotics : the chemicals produced by microorganisms which are able to destroy or inhibit the growth of other microorganisms.

Autotrophic bacteria : these derive the nutritional requirements from inorganic substances of carbohydrates, proteins, vitamins, enzymes etc. like carbon dioxide, carbonates, nitrogen, K_2HPO_4 etc.

Chemosynthetic bacteria : these get their energy from the oxidation of inorganic chemical reactions.

Facultative anaerobes : bacteria which can survive either in the presence or absence of oxygen.

Heterotrophic bacteria : these require one or several preformed organic compounds ranging from a single vitamin to several complex organic compounds.

Micro-aerophiles : bacteria which require oxygen for survival.

Microbial ecology : the study of relationship between microbes and their surrounds (environments).

Modified Atmosphere Packaging (MAP) : the enclosure of food in a package in which the atmosphere has been modified by changing the proportion of various gases.

Obligate aerobes : bacteria which cannot survive in the absence of oxygen.

O-R potential : reducing and oxidizing power of the food.

Osmosis : a biological process in which the water inside the cells move out through the semi-permeable membrane into the concentrated brine.

Plasmolysis : partial dehydration of cells when there is a tendency to equalize water concentration inside and outside the cell.

Plasmoptysis : bursting of a cell when it is placed in distilled water.

Sterilization : a combination of high temperature and time to destroy nearly all microorganisms in foods.

Udder : mammalian glands of the animal from where milk is secreted.

3.8 ANSWERS TO CHECK YOUR PROGRESS EXERCISES

Check Your Progress Exercise 1

1) a) False

   b) True

   c) False

2) a) *Pseudomonas, Chromobacterium, Proteus, Micrococcus, Bacillus, Streptococcus, Enterobacter* and *Escherichia* are present in water.

   b) *Bacillus, Clostridium, Enterobacter, Escherichia, Micrococcus, Alcaligenes, Flavobacterium, Chromobacterium, Pseudomonas, Proteus, Streptococcus, Leuconostoc, Acetobacteria* and *Actinomycetes* are the bacteria present in soil.

3) The common sources of food contamination include the natural microflora associated with the food (plant, animal), the processing equipment, water used, packaging material sewage, air etc.

4) a) *Escherichia coli, Bacillus subtilis, Staphylococcus* are found in milk

   b) *Salmonella, Anthrax, Escherichia coli* are found in meat

   c) *Escherichia coli, Salmonella typhi, Shigella dysenteriae* are found in vegetables and its products.

   d) *Aspergillus* and *Penicillium* are found in fruits

Check Your Progress Exercise 2

1) a) mould, yeast

b) autotrophic, heterotrophic

c) oxidation, sunlight

d) optimum

e) thermophiles, mesophiles, psychrophiles

2) Nutrition, oxygen, temperature, moisture, pH, osmotic pressure and light are the factors affecting growth of microorganism.

3) a) Bacteria which can survive either in the presence or absence of oxygen are referred to as facultative anaerobes.

b) The tendency to equalize water concentration inside and outside the cell causes particle dehydration and shrinkage of cell is referred to as plasmolysis.

c) Osmosis is the outward movement of water through the cell membrane into the concentrated syrup or brine containing 30-40% water.

d) pH is the negative logarithm of the reciprocal of the hydrogen ion concentration.

e) Water activity is the ratio of vapour pressure of the solution to the vapour pressure of pure water.

Check Your Progress Exercise 3

1) a) sundrying

b) lactic

c) pasteurization

d) irradiation

e) radurization, radappertization, radicidation

2) Microorganisms can be destroyed or controlled by the manipulation of the very factors like pH, temperature and water activity, which affect the growth of the microorganisms. This is the principle behind food preservation. Physical or chemical methods are the two methods of food preservation.

3) The different physical methods include pickling, addition of salt and sugar, fermentation, drying, temperature, modified atmospheric packaging.

4) a) 63-65°C for 30 minutes

b) 71.7°C for 15 seconds

c) 132°C for 2 seconds

Check Your Progress Exercise 4

1) a) Salt peter, common salt

b) Chemical

c) GRAS

d) 2.5 - 4.0

e) *Clostridium botulinum*

2) The chemical preservatives act as growth inhibiting or growth retarding agents. Its action includes effect on genetic functions by interaction with DNA molecules, acting on cell wall, inhibition of microbial cell enzymes and making essential nutrients unavailable to the microorganisms.

3) Benzoates, Sorbates, Propionates, Acetates, Nitrates and Nitrities, Sulphur dioxide and sulphites, and Antibiotics are a few GRAS chemicals that are widely used.

4) a) Benzoate / Sorbic Acid

b) Propionate

c) Acetate

d) Nitrates and Nitrites

e) Sulphur dioxide and Sulphites